Kidney International, Vol. 39 (1991), pp. 401–420

Molecular mechanisms of tubulointerstitial hypertrophy and hyperplasia

GUNTER WOLF and ERIC G. NEILSON

Renal-Electrolyte Section of the Department of Medicine, and the Cell Biology and Immunology Graduate Groups, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Aside from the normal, sporadic turnover of epithelial cells along the adult nephron, most somatic cells in the kidney do not divide, or if they are obligated to respond to an external stimulus, there seems to be less hyperplasia in preference for cellular hypertrophy. A variety of pathophysiologic stimuli which provide temporary or permanent fixed reductions in renal function often result, for example, in compensatory enlargement of the kidney, principally in the tubulointerstitium [1]. Such adaptive responses are thought to provide a mechanism for redressing initial damage, as well as providing for a limited restoration of function. The renal hemodynamic alterations associated with these events, like increases in single nephron glomerular filtration rate (SNGFR) and glomerular blood flow, have been extensively reviewed elsewhere [2, 3]. Changes in the size of the tubular nephron, as well as subsequent, late renal fibrosis have traditionally been considered a response to modifications in the mechanics of filtration—the so-called work hypothesis [4]. There is, however, alternative evidence in animals [5, 6], and humans [7], that the cellular processes of compensatory renal enlargement, themselves might share in the responsibility for the continued, long-term loss in remaining nephrons.

Careful assessment of early enlargement parameters, like *de novo* synthesis of phosphatidylcholine, reveal for example, that some biochemical alterations occur earlier than the increase in SNGFR, or can be dissociated from semi-concurrent hemodynamic changes [8, 9]. Recent studies also provide evidence that growth factors can directly modulate renal hemodynamics, consistent with a contemporary view that initial compensatory responses might be responsible for subsequent alterations in hemodynamic parameters [10, 11].

The nature of the adaptive response of nephrons depends on the age of the individual, and whether the underlying initial insult is chronic or more acute. Tubular epithelial cells, for example, respond to acute tubular necrosis with proliferation and mitogenic activity, as defined by an increase in DNA synthesis. The renal growth response in neonatal animal following injury is also hyperplastic. Chronic damage of the adult kidney, in contrast, leans more towards compensatory renal hypertrophy with an increase in size and a rise in the protein/DNA ratio.

© 1991 by the International Society of Nephrology

In the 1960s, the view that approximately 75 to 80% of compensatory renal enlargement in adults was cellular hypertrophy, and the remaining 25 to 20% hyperplasia, is still widely held today [12]. While most anatomical parts of the nephron are probably involved in compensatory hypertrophy, the fact that a major constituent of kidney mass is proximal tubules suggests that an increase in size and protein content of proximal tubular cells may principally contribute to compensatory renal hypertrophy [13]. The present article, therefore, will examine some of these early-onset mechanisms of tubulointerstitial changes. Table 1 gives an overview of currently used experimental models of compensatory renal enlargement. Most models, particularly the ablative ones, result mainly in renal hypertrophy. Others, like the induction of acute tubular necrosis in animals by folate injection, however, are characterized by a mitogenic response producing hyperplasia [1, 14].

Despite the existence of a burgeoning literature dealing with more biochemical aspects of renal enlargement [14–16], the molecular mechanisms involved in the regulation of such changes are not formally understood. Especially incomplete is an understanding of the nuclear and cytoplasmic regulation of genes differentiating compensatory renal hypertrophy from true hyperplasia. Recent discoveries in the fields of epithelial growth factors, signal transduction and gene activation, however, have shed some new light on the molecular mechanisms of cellular enlargement. We will focus on these more recent findings.

Cell cycle

Cells undergoing mitotic divisions can be considered, in a simplified model, as progressing through an orbit of successive phases [17, 18]. These different phases include a stage of enlargement, associated with an increase in protein content, in order to prepare for DNA replication (G_1-phase), DNA replication (S-phase), a post-replication phase of preparation for division (G_2-phase), and finally the mitosis (M) which can be viewed as a cell cycle within itself [19]. The G_1-phase lasts for several hours in many cells with subsequent events seeming to happened sequentially. A poorly defined level of competence in such cells has been traditionally proposed as a requirement for entering the G_1-phase [20]. Competent cells can enter the G_1-phase of the cell cycle without any protein synthesis. They progress towards the S-phase until they reach a special point called V, also named START in budding eukaryotic yeast. After this point rapid protein synthesis is necessary for the further

Table 1. Some in vivo models of renal hypertrophy and hyperplasia

Hypertrophic responses
Renal ablation in adult animals
Unilateral obstruction (contralateral kidney)
High protein intake
Diabetes mellitus (insulin dependent)
Ammonium chloride feeding
Testosterone administration in female animals
Thyroxine administration
Combined hypertrophic and hyperplastic responses
Renal ablation in neonatal animals
Tubular salt overload
Deoxycortocosterone acetate with normal salt diet and furosemide
Potassium depletion
Hyperplasia response
Folate nephropathy
Chemical nephrotoxicity (mercuric chloride, lead acetate)

progression. This part of the G_1-phase may not be dependent on the presence of growth factors [21, 22].

Cell cycle regulation is probably not identical among all species. In eukaryotic yeast, like S. cerevisiae, progression through the cell cycle is controlled at the START point between G_1/S transition, whereas mammalian cells seem to have control points at G_1/S and G_2/M. Recent studies have isolated factors which are involved in the regulation of such control points. MPF (maturation phase promoting factor) is a non-species-specific mitotic inducer which can only be detected in cells undergoing mitosis [23]. The catalytic subunit of MPF is a 34,000 Mr phosphoprotein with kinase activity called pp34 [24]. While originally identified as products of the $cdc2^+$ gene from the fission yeast S. pombe, it has become very clear that other eukaryotic mammalian cells, including humans, have similar genes encoding for proteins homologous to pp34 [25–27]. It is now apparent that pp34 is responsible for the histone H1 kinase activity of MPF which phosphorylates and activates histones H1 [28]. The other subunits of MPF are the cyclins which can be separated into cyclin A and B, sharing 31% amino acid homology [29]. Cyclins accumulate during interphase, and undergo degradation at the end of each mitosis, associated with a drop in MPF activity [30].

The complex regulation of MPF has become more clear with recent experiments in yeasts and in various mammalian tissues [31]. S. pombe needs $cdc2^+$ activation for passage through START, and for the induction of mitosis [24]. The control protein pp34 is inactive when its is phosphorylated. During mitosis threonine and tyrosine residues of pp34 are dephosphorylated resulting in increased activity of pp34 [32].

The factors which regulate the phosphorylation of pp34 are unknown, but in yeast, expression of the gene cdc25 activates pp34 [33], whereas the product of the wee1 gene, which has homology with other protein kinases, inhibits pp34 [34]. The level of pp34 is constant during the cell cycle, whereas cyclin must accumulate during interphase. Cyclin forms with phosphorylated, inactive pp34 as a complex that still has no MPF activity [18]. Dephosphorylation of pp34 and phosphorylation of cyclin is necessary for MPF activity and entry into mitosis. After activation of pp34, through dephosphorylation, probably pp34 itself phosphorylates cyclin. Possible substrates for pp34 include H1 histones, lamins, nucleolin, and $pp60^{src}$ [17]. Phosphorylation of lamins is transient (only during mitosis), and is required for the breakdown of the nuclear envelope and reorganization of the cytoskeleton during mitosis [17]. Activated MPF induces the degradation of cyclin leading to the return to the interphase [18]. The regulation of cyclin degradation is complex and may involve a factor called IMF (inhibitor of MPF) [18, 35]. It has been suggested that in a similar fashion as at the G_2/M transition, pp34 interacts with a cyclin-like molecule to induce protein kinase activity at START, the control point of G_1/S transition [18]. Thus, all major control points of the cell cycle are regulated by an interaction between pp34 and different cyclins, in which the activation of pp34 through dephosphorylation is a key step in the regulation of cycling events [36].

The majority of renal tubular cells do not normally undergo repeated rounds of cell duplication. They rather remain in a quiescent state, with a normal complement of DNA (G_0-phase/2n), where they carry out their multiple biochemical cell functions [22]. The G_0-phase can be recreated in cell culture through deprivation of serum mitogenic factors. After the addition of serum, or serum-derived growth factors, many cells in the G_0-phase re-enter the cell cycle and resume proliferation. In vitro studies have shown that, shortly before the onset of the S-phase, cell cycle events become independent of growth factors in the remaining phases of the cycle. In mitogenic renal growth responses, for example after acute tubular necrosis, tubular epithelia begin to enter from the normal G_0-phase of the cell cycle, replicating their DNA in the S-phase and dividing. The result is hyperplasia regeneration. In renal hypertrophy, to the contrary, cells entering from the G_0-phase, do not progress to DNA-replication (S-phase). The mechanisms of arresting cells in the G_1-phase, that is, keeping pp34 phosphorylated [36], are unclear. Possible events which might contribute to such a process will be described below.

Tubulointerstitial environment

Several types of cells, including tubular epithelium, a variety of interstitial cells (Type I and II in the cortex, types I to III in the medulla), interstitial fibroblasts, vascular endothelium, macrophages and several phenotypes of lymphocytes, constitute the cellular registry of the tubulointerstitial microenvironment [37]. While tubular cells located along the nephron are known to be discretely differentiated in various phenotypes, recent findings also suggest that interstitial kidney fibroblasts are a distinct population of cells adapted to the tubulointerstitial microenvironment. These kidney fibroblasts have growth properties and biosynthetic characteristics of matrix proteins which can be distinguished from syngeneic dermal fibroblasts [38–41]. Activated fibroblasts also secrete cytokines which are mitogenic for tubular cells [41], and likewise, tubular cells release proliferative cytokines for fibroblasts, like insulin growth factor [42]. Resident macrophages and lymphocytes localized in the tubulointerstitium can secrete cytokines like interferon, interleukins (IL-1-6), platelet-derived growth factors (PDGF), tumor necrosis factor (TNF), transforming growth factor alpha (TGFα) and transforming growth factor beta (TGFβ) [43, 44]. Somatic cells of the tubulointerstitium can be immune regulated by mononuclear cells [43, 45]. Cytokines like interleukin 1 (IL-1) and TNF are also important modulators of vascular endothelial growth [46]. Endothelial cells, themselves, are capable of synthesis and secretion of IL-1, PDGF, granulocyte-

macrophage colony stimulating factor (GM-CSF), interferon-alpha, and TGFβ which, in turn, can modify the proliferative response of other cells located in the tubulointerstitium [46–48]. Thus, close interactive effects between different cell types, sharing a complex cytokine network in the tubulointerstitial microenvironment, can readily modify the rate of cell division and size of their phenotypic neighbors.

Growth factors

Dating back into the fifties [49] and even more recently [50], attempts have been made to characterize specific regulators of renal hypertrophy circulating in the plasma, particularly after uninephrectomy. These substances have been called renotropins. Although there is evidence that such factors might exist, the results of these studies are somewhat inconclusive. Some of these factors, for example, may be similar to the polypeptide growth factors described in detail below. The isolation, purification and cloning of growth factors and their receptors, however, along with new insights into the signal transduction pathways coupled to those receptors, have provided a contemporary knowledge regarding cellular and molecular mechanisms of renal enlargement.

There is increasing evidence for a physiological and/or pathophysiological function for such cytokines in renal enlargement [51, 52]. Tubular cells can secrete several of these growth factors and they, and other renal interstitial cells, have receptors for many of these factors [53, 54]. Therefore, the local release of growth factors may modulate tubular enlargement in an autocrine and/or paracrine manner. Moreover, a change in number or affinity of growth factor receptors, the interaction with cytokines released by monocytes/macrophages, fibroblast and endothelium, the endocrine circulation of growth promoting hormones and finally, the local degradation of these factors collectively create a complicated interactional picture [55]. Although the establishment and culture of several tubular cell lines in serum free medium has promoted the investigation of growth factor effects on cellular proliferation and hypertrophy [56, 57], the contributions of a single growth factor to the whole framework of renal enlargement in vivo remains to be established. In defined serum-free media, however, some growth factors, including epidermal growth factor (EGF), PDGF, and TGFα are mitogenic [51, 57], and induce proliferation in cultured tubular cells, while other factors such as insulin-like growth factor I (IGF-I), insulin, TGFβ, prostaglandin E_2, and angiotensin II have been reported to preferentially induce cellular hypertrophy [58–60]. Most growth factors probably mediate the transition from G_0 to G_1 in quiescent cells.

Platelet-derived growth factor (PDGF)

Human PDGF is a heterodimer of A (14 to 18,000 Mr) and B (16,000 Mr) chains linked together by disulfide bridges [61]. Both chains are synthesized as larger precursors and subsequently processed into functional moieties [62, 63]. The PDGF-B chain is nearly identical with the product of the c-sis oncogene [64, 65]. The extracellular domain of the PDGF-receptor consists of 80 to 100 amino acids resembling immunoglobulin domains in its secondary structure. Two different genes for PDGF-receptors have been described whose products demonstrate different responses to the PDGF subtypes [66]. Although PDGF is normally released from alpha-granules in platelets [65], recent findings indicate that PDGF is produced by epithelial kidney BSC-1 cells, macrophages/monocytes and endothelium [67, 68]. Adenosine diphosphate is also able to stimulate c-sis expression and release of PDGF in that system. Interestingly, BSC-1 cells do not respond to PDGF in vitro with mitogenesis, implying perhaps a paracrine mechanism of PDGF-mediated proliferation. PDGF is mitogenic for kidney endothelial and epithelial cells, and renal fibroblasts derived from the tubulointerstitium. PDGF, furthermore, can stimulate the release of other growth factors like IGF-I from fibroblasts [65].

Epidermal growth factor (EGF)

Mouse EGF is synthesized as a prepro-precursor molecule of 1217 amino acids which is inserted into the membrane as a transmembrane protein [69–71]. The cellular c-mos oncogene shares sequences with pro-EGF. Specific proteases release the active factor resulting in a single chain polypeptide of 53 amino acids [72]. Human and mouse EGF have about 70% amino acid homology [73]. The main sites of EGF synthesis in adult mice are the kidney and the salivary gland, but other tissues might as well produce EGF [70]. More than 90% of urinary EGF activity is derived from renal synthesis [74, 75]. EGF immunoreactivity, and in prepro-EGF mRNA transcripts, using in situ hybridization, are localized to the thick ascending limb of Henle and the distal convoluted tubule, but not to the proximal tubule [76–78]. Ultrastructural investigation shows the presence of EGF in luminal membranes and apical vesicles [79]. Specific receptors for EGF have been demonstrated in the basolateral membrane of the proximal tubule and in a variety of different cell lines originating from the proximal tubule [80, 81]. The EGF-receptor is a 170,000 Mr protein with intracellular tyrosine kinase activity [82]. Interestingly, thyroid and growth hormone can induce hepatic EGF-receptors, providing one mechanism for how endocrine hormones can modify the action of local growth factors [83, 84]. In contrast, interferon α downregulates EGF receptors on Madin-Darby bovine kidney cells, explaining in part the growth inhibitory effects of interferon [85]. EGF is mitogenic for tubular epithelial cells in culture [57, 86]. Moreover, EGF can inhibit gluconeogenesis in proximal tubules [81].

Renal ischemia reduces prepro-EGF mRNA levels, but increases the number of EGF receptors [87]. Infusion of EGF enhances recovery from acute tubular necrosis [88]. These findings indicate that intra-renal EGF might modulate the mitogenic response after acute tubular necrosis. Immunostainable EGF in the apical cell parts of the distal tubule also continuously increases up to two weeks after uninephrectomy [89]. An increase in immunoreactive EGF and prepro-EGF mRNA transcripts were observed in the remnant kidney seven days after uninephrectomy, indicating that increases in intra-renal EGF synthesis is a rather late event in the time course of compensatory renal growth [90]. Binding of EGF to basolateral tubular membranes was decreased three weeks after uninephrectomy, which might likely indicate a down regulation of its receptor [91]. Mice made deficient of circulating EGF by sialoadenectomy also fail to undergo compensatory renal growth after unilateral nephrectomy. Exogeneous EGF replacement restored the growth response [92]. Other investigators, however, have found no difference in EGF receptor density between controls and rats receiving uninephrectomy [93]. The

hyperplastic regeneration of tubules following folic acid administration was associated with an upregulation of EGF receptors [93]. Since EGF is a mitogen in vitro for tubular epithelial cells, the real role of EGF in renal hypertrophy remains uncertain.

Insulin-like growth factors (IGFs)

IGFs share approximately 50% homology with pro-insulin. IGF-I (Somatomedin C) and IGF-II (Somatomedin A) have 62% homology [94]. Both factors are monomeric polypeptides which are cleaved from larger precursors. IGF-I contains 70 amino acids whereas IGF-II is composed of 73 amino acids [95]. The human IGF-I gene consists of five exons localized on chromosome 12 [96]. The IGF-II gene is composed of seven exons and maps to chromosome 11 [97]. Alternative splicing of mRNAs of both genes occurs. IGF-I is produced in many tissues, including the kidney where it localizes to mesangial cells and principal cells of the collecting duct [98, 99]. The production of IGF-I is stimulated by pituitary growth hormone (GH) [100]. IGF-II mRNA can also be detected in the kidney and may be involved in growth regulation of the developing fetal nephrons [101]. The receptor for IGF-I (type I IGF receptor) consists of two peptide-binding alpha-subunits and two beta-subunits with tyrosine kinase activity [102]. The IGF-I receptor and the insulin receptor share 80% identity in the tyrosine kinase domain. The receptor has three times lower affinity for IGF-II and a low affinity for insulin [103]. Peptide binding to the receptor results in autophosphorylation of the beta-subunit. A second IGF receptor (type II IGF receptor) is present in almost all cell types. This receptor has a higher affinity for IGF-II than IGF-I, and also does not bind insulin [104]. The type II IGF receptor shares a high sequence homology with a membrane lectin-like receptor for mannose-6-phosphate, and consequently, can bind proteins with mannose 6-phosphates, although only at a different molecular site than IGF [105]. The IGF-II receptor is a single peptide with an extracellular domain connected through a transmembrane segment to a cytoplasmic region composed of 164 amino acids without any similarity to known sequences. IGF-II probably mediates its mitogenic effects through the type I IGF receptor, since antiserum against the IGF-II/mannose 6-phosphate receptor can not block mitogenic activity of IGF-II. IGF-I receptors which undergo autophosphorylation after IGF-I binding have been detected on the basolateral membrane of the proximal tubule [106]. In addition, IGF-I receptors with several-fold less binding capacity, and without signal transduction activity are localized in luminal membranes of the proximal tubule. IGF-II receptors are also localized in basolateral and brush border membranes [107]. IGF-I has also been found in regenerating S3 segments of the proximal tubule after acute ischemic injury in rats [108]. The immunoreactivity of IGF-I decreases and finally vanishes with increasing differentiation of regenerating cells. Stiles and co-workers demonstrated an increase in immunoreactive IGF-I extracted from whole kidneys five days after uninephrectomy [109]. In hypophysectomized animals, the increase of IGF-I was of the same magnitude, but from lowered basal IGF-I levels. In another study, an increase of kidney extracted IGF-I of 58% over controls was found after 24 hours. A similar increase of IGF-I was demonstrated in rats made diabetic by streptozotocin treatment [110], and 24 hours after uninephrectomy in Wistar rats [111]. Feeding the rats a low (8% casein), but not a medium or high (78% casein) protein diet before nephrectomy, blunted the increase of IGF-I in the remnant kidney. Renal IGF-I content was not different among sham-operated animals on the different diets. A report by the same group showed that dwarf rats deficient in GH increase their acid extractable renal IGF-I and undergo compensatory renal enlargement after uninephrectomy [112]. More recently, a fivefold increase in mRNA transcripts for IGF-I, using dot-blot hybridization, was observed 24 hours after unilateral nephrectomy in the rats [113]. This increase was sustained for one week and occurred in the absence of any changes in circulating IGF-I or GH levels. In contrast, using a solution-hybridization nuclease-protection assay, no changes in steady-state IGF-I mRNA levels could be detected between controls or nephrectomized animals [114]. However, an increase of immunoreactive IGF-I in collecting ducts was observed five days after uninephrectomy, but not after one or two days [114]. PDGF and fibroblast growth factor (FGF) stimulate the release of IGF-I/somatomedin C from fibroblasts in vitro [115]. IGF-I itself is only a weak mitogen for fibroblasts, but can act synergistically with EGF to induce proliferation [116]. Furthermore, fibroblasts produce different IGF-binding proteins [117]. The production of these proteins is also under hormonal control. The possibility that fibroblasts secrete and respond to IGF-I and IGF-binding proteins, in an autocrine/paracrine manner, indicates the potential of complex interactions between tubular epithelium and fibroblast. Considering all these finds, there is reasonable evidence that IGF-I plays some role in the process of compensatory renal enlargement.

Transforming growth factors (TGFs)

Transforming growth factor α (TGFα), a 50 amino acid peptide, shares 40% sequence homology with EGF [118, 119]. TGFα binds to the EGF-receptors and phosphorylates tyrosine residues [120]. TGFα is able to induce autosynthesis as well as activation of protein kinase C-induced synthesis of TGFα. Transmembrane forms of TGFα can activate the EGF-receptor and might play an adjunctive role in cell-cell contact and interactions between different types of cells [121]. Although initially found in virally transformed cells, there is increasing evidence that TGFα is also produced by non-transformed cells. For example, TGFα is synthesized and secreted by activated macrophages [122]. BSC-1 cells seem not to produce TGFα, whereas normal human kidney express mRNA transcripts for TGFα [51]. Recent preliminary data applying rabbit anti-TGFα antisera to histologic tissues have immunolocalized TGFα to cortical collecting ducts of the rat kidney [123]. Intra-aortic infusions of TGFα accelerate renal repair and recovery after experimental ischemic injury to the kidney [124]. Whether TGFα plays any role in the physiological regulation of renal enlargement is unknown.

TGFβ is a homodimer consisting of two disulfide-linked chains of 12,500 Mr, and is not related to TGFα [125–128]. TGFβ1, originally purified from platelets and kidney [127, 128], is virtually produced by all cells. More recently, TGFβ2, with a different N-terminal amino acid sequence, and heterodimers (TGFβ1.2) consisting of one TGFβ1 and one TGFβ2 chain, have been described [126]. Monkey BSC-1 tubular cells in culture secrete TGFβ2 [129]. TGFβs, similar to other polypeptide growth factors, are synthesized as larger secretory precursors which are then cleaved into smaller fragments [130].

TGFβ2 and TGFβ1.2 have probably a more restricted tissue distribution than TGFβ1. The TGFβs are members of a larger family of growth inhibitory factors [130, 131].

Two distinct types of TGFβ receptors have been characterized: the type I TGFβ receptor is a disulfide-linked complex of 565 to 615,000 Mr with a Kd of 50 to 500 pM, and the smaller type II TGFβ receptor with a Kd of 50 pM and a molecular weight of 85 to 110,000 Mr [132, 133]. Although many tissues seem to have both types of receptors, only the type II receptor is expressed in rat myoblasts. The functional difference between the receptors is not known. TGFβ receptors have no tyrosine activity and are not down regulated after ligand binding. TGFβ has complex effects on the proliferation and differentiation of cells [134]. TGFβ can either stimulate or inhibit proliferation depending on the cell type, growth conditions and presence of other growth factors. TGFβ stimulates the proliferation of AKR-2B cells in monolayers, and fibroblasts in soft-agar [135]. Treatment of AKR-2B cells with TGFβ stimulates the early induction of c-sis and the corresponding secretion of a PDGF-like factor into the supernatant. Moreover, immediate early gene expression is also induced with coculture. These data suggest that TGFβ mediated mitogenesis in this cell line occurs through the induction of c-sis with subsequent autocrine proliferation through PDGF. Where the proliferation of epithelial cells is concerned, including renal proximal tubular cells, TGFβ has mainly inhibitory effects [58, 129]. TGFβ, insulin, and hydrocortisone together induce hypertrophy in BSC-1 cells, whereas insulin and hydrocortisone alone are mitogenic for this cell line, suggesting that the presence of TGFβ, in culture, transfigures proliferative growth into hypertrophy [58]. In the culture of proximal tubule cells, TGFβ promotes a morphologic change from monolayers into solid clusters of cells [136]. The change in appearance of cells is associated with alterations in the composition of the extracellular matrix [136]. TGFβ also stimulates transcription, synthesis, and secretion of procollagens type I, III, IV and fibronectin [137, 138]. Furthermore, TGFβ inhibits matrix degradation by inhibiting the secretion of proteases as well as increasing the synthesis of protease inhibitors. TGFβ can also directly stimulate the activity of the mouse α2(I) procollagen promoters as well as the fibronectin promoter [139]. In addition, TGFβ may have effects on the post-translational levels of collagens. TGFβ increases the expression of cell membrane receptors specific for cell adhesion proteins linking the extracellular matrix to the cytoskeleton of the cell [140]. These changes of the extracellular matrix might facilitate hypertrophic enlargement and the arrest of proliferation.

Fibroblast growth factors

Acidic fibroblast growth factor (aFGF) and basic FGF (bFGF) are members of a large family of related proteins expressed in different tissues [141]. FGFs are strongly bound to heparin and heparan sulfate. Basic pituitary FGF is a 146 amino acid protein. Four molecular forms of bFGF can be co-expressed from one mRNA, in a hepatoma cell line by starting translation at non-AUG codons [142]. bFGF isolated from kidney shares homologous sequences with pituitary FGF. Recently a cDNA sequence of the 130,000 Mr receptor for bFGF was isolated from Swiss 3T3 cells [143].

Human aFGF is 155 amino acids in length. The coding region for aFGF is interrupted by two introns [144]. Kidney FGF is mitogenic for endothelial cells, smooth muscle cells, and fibroblasts. Although FGF has been shown to be mitogenic for some epithelia, it is not known whether kidney FGF plays any role in the autocrine/paracrine growth regulation of tubular cells. FGF, however, might be involved in angiogenesis and differentiation during renal organogenesis [145].

Ammonia

It has been long known that chronic metabolic acidosis induced in rats by feeding them NH_4Cl can lead to renal hypertrophy [146]. An increase in the urinary excretion of acid equivalents, induced by the metabolic acidosis, has been considered to be largely responsible for the renal hypertrophy, according to the working hypothesis [147]. However, a recent study demonstrated that NH_4Cl added to the medium of cultured rabbit proximal tubule cells can directly induce cellular hypertrophy in the absence of extracellular acidemia even when the Na^+/H^+ antiporter is inhibited by amiloride [148]. An increase in protein synthesis and a decrease in protein degradation were the cause for the NH_4Cl induced hypertrophy. Although the mechanisms of NH_4Cl on protein synthesis and turnover remained to be established, this study suggests that an increase in renal ammoniagenesis under special conditions in vivo might directly stimulate renal hypertrophy rather than the metabolic acidosis itself.

Other growth factors

Reducing extracellular potassium concentrations in cell culture or feeding rats a potassium-deficient diet can induce cellular proliferation and activation of glyceraldehyde-3-phosphate dehydrogenase [149]. In elegant studies, Toback and co-workers demonstrated that these effects are due to an unknown mitogenic factor released by stimulated cells in an autocrine manner [150]. Furthermore, a reduction in sodium concentration in the culture medium to 130 mM stimulated proliferation of monkey kidney epithelial cells (BSC-1 cells), but not fibroblasts. BSC-1 cells released autocrine growth factors with the molecular weight of 6,200 and 9,000 Mr after exposure to low concentrations of sodium [151, 152]. The low sodium-induced growth factors are not identical with hitherto characterized factors. Angiotensin II (Ang II) induces hypertrophy, but not mitogenesis in a proximal tubular cell line [60]. FACS analysis revealed that Ang II-treated cells remained in the G_1-phase of the cell cycle. Adenosine diphosphate is another strong mitogenic stimulus for BSC-1 monkey kidney cells [153]. Table 2 shows that cytokines released from macrophages/lymphocytes have at least in vitro modulatory effects on growth and collagen secretion in a tubular epithelial cell lines. Tamm-Horsfall glycoprotein, localized to the ascending limb of Henle's loop, can also bind lymphokines like IL-1 [154]. Whether cytokines released from tubulointerstitial macrophages/lymphocytes play any role in normal renal growth in vivo is unclear.

Proto-oncogenes in growth regulatory mechanisms

The transforming oncogenes of retroviruses have homologous counterparts in normal cells [155]. These proto-oncogenes appear to play a pivotal role in the regulation of eukaryotic growth and differentiation [156, 157]. Proto-oncogene products

Table 2. Effect of different cytokines on the cell cycle and collagen secretion of cultured murine proximal tubule cells (MCT cells)

Cytokines	Collagen types I	III	IV	V	Proliferation
Interleukin I	−−	+	−	−	+/−
Interleukin II	−−	−−	−−	−−	−
EGF	++	+/−	+/−	n.i.	+++
PDGF	++	n.i.	++	n.i.	+
TGFβ	−	+	+	+/−	−−−
γIFN	n.i.	n.i.	n.i.	n.i.	−
Angiotensin II	+/−	n.i.	++	n.i.	(hypertrophy)

Abbreviations are: +++, strong stimulation; ++, medium stimulation; +, weak stimulation; +/−, no change; −, weak inhibition; −−, medium inhibition; −−−, strong inhibition; n.i., not investigated.

Table 3. Oncogenes expressed during epithelial hypertrophy and/or hyperplasia

Oncogenes encoding for growth factors (Signals)	
c-sis	encode for the B-chain of PDGF
Oncogenes related to growth factor receptors (Receptors)	
c-erb B	encode for a truncated version of the EGF receptor with tyrosine protein kinase activity
c-neu (c-erb B-2)	a transmembrane protein with tyrosine kinase activity
c-ros	the oncogene product shares more than 70% homology with the tyrosine protein kinase domain of the insulin receptor
c-mas	relationship to angiotensin receptor
c-mes	transmembrane protein with tyrosine kinase activity
c-fms	receptor for colony-stimulating factor on monocytes and tissue macrophages
Oncogenes with protein kinase activity without transmembrane domain (Transducers)	
c-src	non transmembraneous tyrosine-specific protein kinase (p60src)
c-mos	serine/threonine protein kinase
Oncogenes related to G-proteins (Modulators)	
c-H-ras-1	ras encoded proteins of c-Ki-ras 2 approximately 21 kD (p21ras) c-N-ras bind guanine nucleotides and have intrinsic GTPase activity
c-rho	share 35–85% with ras genes
Nuclear oncogenes (Targets)	
c-fos	c-fos protein forms with the c-jun a heterodimer through a leucine zipper domain. The heterodimer transactivates sequence-specific promotor regions of DNA.
c-myb	highly conserved 75 kD nuclear DNA binding protein expressed mainly in hematopoietic cells
c-myc	62–64 kD nuclear phosphoprotein with DNA-binding properties

act at different points in this complex growth control network (Table 3). Proto-oncogenes can be grouped into different families: some encode for growth factors like the c-sis oncogene which produces the B-chain of PDGF; others, like c-erb A, c-erb B, and c-neu, are related to growth factor receptors with tyrosine kinase functions [155, 158]. Additional oncogenes like c-src and c-abl are related to oncogenic tyrosine kinases lacking a transmembrane domain, and are probably involved in the intracellular transduction of signals [159]. A group of oncogenes (Ha-, Ki- and N-ras) encode for GTP-binding proteins with GTPase activity, and might act as coupling factors in signal transduction. Finally, distinct nuclear oncogenes, like c-myc, c-fos and c-jun, are activated by many growth stimuli [157]. Some of these oncogenes encode proteins which, acting together, are fundamental transacting factors in the regulation of other genes [157, 158]. They are also necessary for the re-entry of rested cells from the G_0 phase into the cell cycle [21].

Signal transduction pathways

Figure 1 gives an overview of signal transduction pathways which might be involved in renal growth regulation. Each cell type has a distinct pattern of receptors responding to growth factors. The distribution of receptors, as indicated above, for special growth factors along the nephron is heterogeneous. Many of these receptors for growth factors share common structural motifs [155], while curiously enough, the growth factors themselves usually have quite diverse structures. While such receptors can be arbitrarily distinguished by whether they are coupled to GTP-binding proteins (G-proteins) or by whether they have cytoplasmic tyrosine kinase activity, some receptors seem to activate pathways that include both G-proteins and protein kinases [157].

GTP-binding proteins (G-proteins) and cAMP

G-proteins are intracellular modulators that carry signaled information from activated membrane receptors to effector enzymes, or ion channels [160]. Most extensively studied is the adenylate cyclase system which is controlled by a stimulatory G-protein (G_s) and inhibitory G-protein (G_i) [161]. By linking G_s and G_i to different receptors, adenylate cyclase can be stimulated or inhibited as a result of ligand binding to a receptor [162]. G-proteins are heterodimers consisting of three polypeptides: a GTP binding α-chain (39 to 52,000 Mr), a β-chain (35 to 36,000 Mr), and a γ-chain (8,000 Mr) which are membrane anchored, and contribute to the receptor recognition site [163]. At the present time nine genes encoding for α-subunits and two for the β-subunits have been isolated [160, 164]. Smaller GTP binding proteins of 20 to 24,000 Mr are also products of c-ras oncogenes [165].

Activation of a membrane receptor causes G-protein-receptor interaction and accelerates the exchange of GTP for GDP which is bound by the α-subunit of the oligomer [166]. A lowering of the Mg^{++} concentration is necessary for this reaction step. Binding of GTP to the α-subunit results in a release from $G_{\beta\gamma}$ chains, and subsequent alterations of the target effector. In the termination step, GTP is hydrolyzed to GDP, the α-subunit dissociates from the effector and reassociates with βγ-subunit [160–163]. Cholera toxin enhances adenylate cyclase activity through ADP-ribosylation of the α-subunit

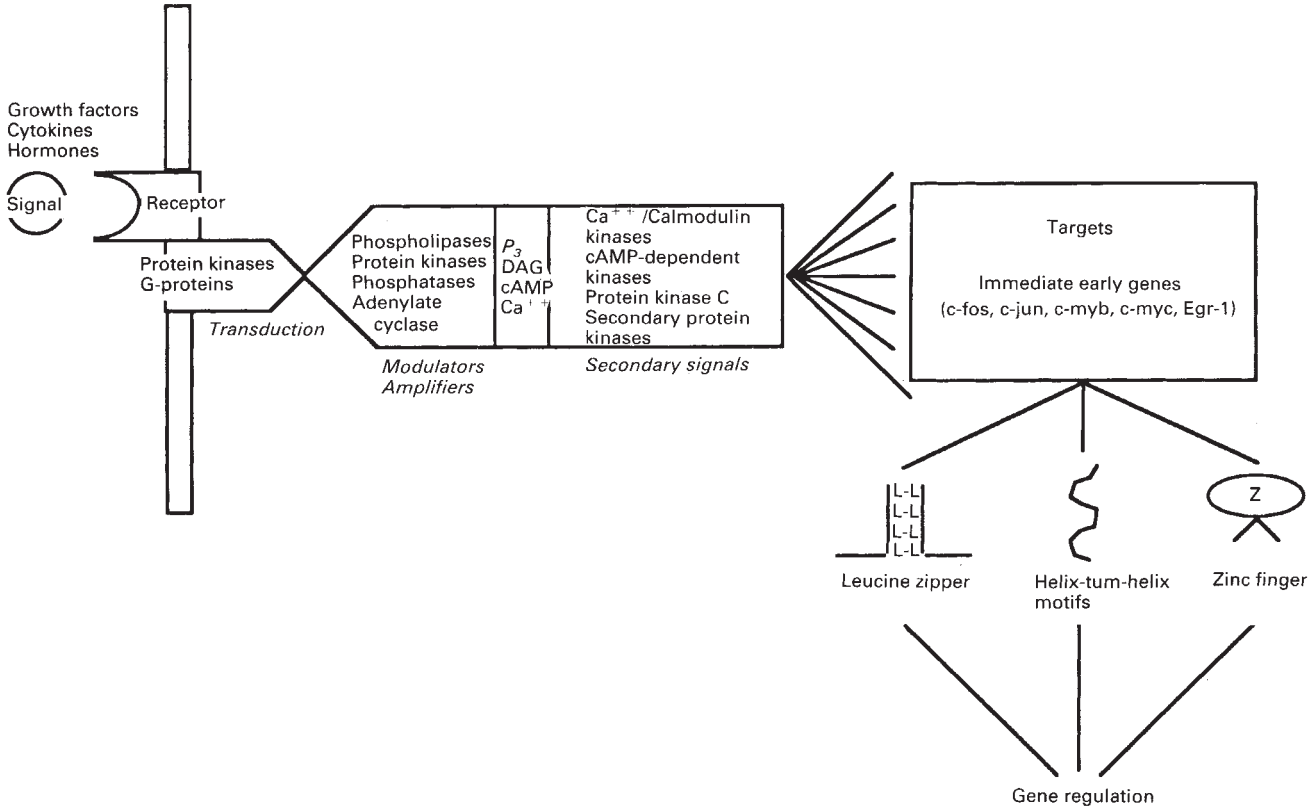

Fig. 1. *A simplified schematic of signal transduction pathways involved in the regulation of hypertrophy and hyperplasia.* After a growth factor or cytokine binds to its receptor, a signal is transduced by G-proteins and/or protein kinases. Modulators and amplifiers of these effects can be cAMP, IP_3, DAG, or Ca^{++}. Secondary signals are expressed by the activation of protein kinases like protein kinase C, tyrosine kinases or cAMP-dependent kinases which transmit signals or phosphorylate protein factors that interact with promoter/enhancer regions of immediate early genes. In the cascade of differential phosphorylation of target-proteins, phosphatases, which themselves are tightly regulated, might also play a pivotal role. Protein products of early genes can act through leucine zipper, zinc fingers domains or helixmotifs as transacting factors for the activation or repression of subsequent genes. Several signal pathways might simultaneously operate in parallel in or a non-linear fashion producing cross-talk between the different systems.

of G_s [167, 168]. In contrast, pertussis toxin interacts with cysteine residues of G_i by ADP-ribosylation, intercepting the tranduction of inhibition to adenylate cyclase. Increasing levels of cyclic AMP (cAMP) are mitogenic for 3T3 cells in synergy with other growth factors [169]. PDGF can also induce an accumulation of cAMP in fibroblasts. In the same cell type, a stimulation of protein kinase C results in an increase in cAMP in the presence of forskolin or cholera toxin [170, 171]. An elevation of intracellular cAMP levels induces the phosphorylation of vimentin, an intermediate filament protein of 58,000 Mr, but not the phosphorylation of protein kinase C [172]. Recent findings indicate that cAMP can regulate gene expression through a 43,000 Mr transacting factor which binds to a 30 nucleotide cAMP response element (CRE) [173]. The purified protein is phosphorylated by cAMP-dependent protein kinase in vitro. In a model system of adenovirus transfected pheochromocytoma PC12 cells, cAMP-dependent protein kinase activates early E2a and E3 promoters [174]. CRE binding proteins recognize the sequence cassette TGACGTCA, present in various promoters including c-fos. Moreover, cAMP induces the transcription of its own degrading enzyme, phosphodiesterase [175]. PTH and magnesium-dependent adenylate cyclase activity in cortical membranes from kidneys with compensatory renal enlargement are significantly lower than in controls [176]. A decrease in the activity of the α-subunit from G_s was detected by cholera toxin-mediated ADP-ribosylation, suggesting alterations of G_s in compensatory renal enlargement [176].

Inositol-phosphatidyl systems

Many polypeptide growth hormones stimulate membrane bound phospholipase C [177] which hydrolyzes phosphatidyl inositol biphosphate to inositol triphosphate (IP_3) and diacylglycerol (DAG). Growth hormone binding to its specific receptor activates phospholipase C in basolateral renal membrane preparations from canine proximal tubule [178]. There is increasing evidence that the receptor-mediated activation of phospholipase C is transduced by a G-protein [179]. In many cells, growth factor receptor-mediated phosphoinositide hydrolysis is insensitive to pertussis or cholera toxin treatment [180]. In other tissues, however, pertussis toxin blocks hydrolysis of inositol biphosphate and the increase in intracellular Ca^{++} [181, 182]. The involvement of c-ras encoded proteins, as G-proteins, associated with the phospholipase C system has been emphasized in recent reports [183]. The ras proteins belong to a family of closely related peptides known as p21 which are present in all eukaryotes [184]. Three ras genes

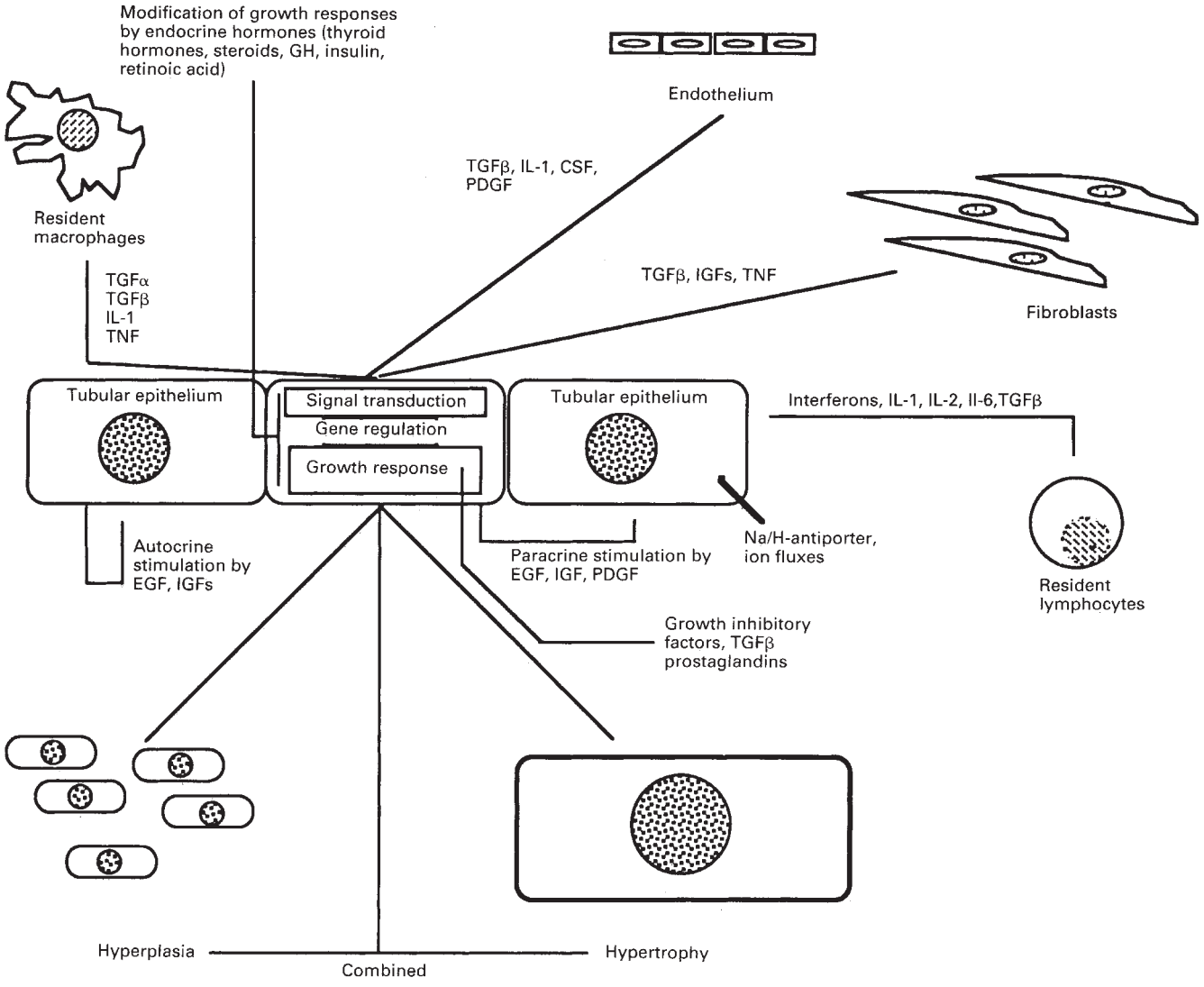

Fig. 2. *Summary of events characterizing the hypertrophic or hyperplasic response of tubular cells in the tubulointerstitial microenvironment.* Pathophysiologic mechanisms, such as those described in Table 1, might lead to autocrine or paracrine stimulation of tubular epithelial cells. Other cell types resident in the tubulointerstitium, like fibroblasts, endothelium, resident macrophages and lymphocytes may all also participate and modify the epithelial response through a complex cytokine network. Such cytokines might also have proliferative effects on tubular cells like EGF, PDGF, or alternatively inhibit mitogenesis as in the case of IL-2 or TGF-β [41, 57, 86]. Additional factors like IGF and angiotensin II can induce cellular hypertrophy in the absence of proliferation [60, 109]. Local actions of cytokines are under the influence of endocrine hormones which modify local cytokine receptors expression, activation of Na^+/H^+ antiporters as well as modify the general susceptibility of resident cell responses to growth factors and cytokines. The specific or pivotal action of a single cytokine in this complicated network in vivo, however, is difficult to establish, since the majority of data has been generated in cell culture systems applying single factor models. The exact selecting mechanisms determining the degree of proliferation versus hypertrophic enlargement of renal cells are unknown, but might involve growth inhibitory factors like TGF-β or prostaglandins [1, 41, 58, 129].

(H-ras-1, K-ras-2, and N-ras) and two pseudogenes (H-ras-2 and K-ras-1) have been identified [185]. Some mouse strains have additional ras pseudogenes. Ras genes are members of a highly conserved superfamily of genes, and other new classes of ras related genes (rho) sharing 30 to 40% homology have been characterized recently. Four exons of ras encode for p21. K-ras-2 has two alternative fourth exons encoding for two isomorphic forms of p21 with slightly different carboxyterminal domains. Studies with a neutralizing anti-ras antibody revealed that p21 proteins are necessary for the initiation of the S-phase of the cell cycle, whereas the expression of c-ras oncogenes are at their highest level in the middle-to-late G_1-phase. Recently the association of ras proteins, as transducers of phospholipase C, have been questioned, since studies applying antibodies against ras proteins in transformed cells demonstrate that ras proteins are not directly involved in the regulation of phospholipase C [186]. Purification and cloning of genes for phospholipase C has revealed a rather unexpected complexity. Different tissues have phospholipase C enzymes of different molecular weights, suggesting multiple isoenzymes [187]. At the present

time four different genes for phospholipase C have been identified. Phospholipase C from rat kidney has a molecular weight of 90,000 Mr [188]. Stable association of the PDGF-receptor with phospholipase C and tyrosine phosphorylation of the enzyme occurs after PDGF binding to its receptor [189]. IP_3 mobilizes intracellular Ca^{++}, which can then bind to calmodulin, and subsequently activates calmodulin-dependent enzymes [190–192]. DAG activates protein kinase C (PKC), which has a high-affinity binding site for mitogenic phorbol esters [193]. PKC can bind to phorbol ester [194, 195]. Chronic treatment with phorbol ester also leads to a decrease in specific binding sites, and a decrease in PKC activity with a subsequent desensitization of cells for growth stimuli [196]. On the other hand, stimulating PKC by phorbol ester might lead to a phosphorylation of phospholipase C or the involvement of G-protein, resulting in unresponsive receptor stimulation. The pivotal role of PKC in mitogenesis was additionally suggested by the proliferative effect of synthetic 1-oleoyl-2-acetylglycerol [195]. Molecular cloning as well as enzymological analysis has shown a molecular heterogeneity of PKC in various tissues [197]. Alpha, beta I, beta II, delta and theta subspecies are expressed in many tissues, whereas gamma and epsilon are expressed only in brain cells [197, 198]. Interestingly, the amino-terminal domain of PKC contains repeats of cysteine-rich sequences resembling zinc-finger motifs found in DNA-binding proteins [199, 200].

There is hitherto, however, no evidence that PKC directly binds to DNA. The kidney contains alpha, beta I and beta II subspecies of PKC. PKC is found in tubular membranes [201]. In vitro studies suggest that DAG can bind to cytosolic PKC, inducing a shift to a membrane-associated form [202]. Other findings reveal, however, that the cytosolic 50,000 Mr fragment of PKC can phosphorylate proteins. This indicates that an association with the membrane is not necessary for function. A variety of growth factors, including PDGF [203] and vasopressin [204], activate PKC in quiescent 3T3 fibroblasts, whereas EGF and insulin do not stimulate PKC activity in these cells. Activation of PKC is reflected by the phosphorylation of a cellular protein with a molecular weight of 85,000 Mr [205]. PKC activity of brush border membranes, but not basolateral membrane, from rat proximal tubular cells was significantly increased 24 hours after unilateral nephrectomy [206] compared to controls. Such findings suggest an involvement of the IP_3/DAG pathway in compensatory renal enlargement. A 100% increase in the concentration of DAG has been reported in remnant kidney cortical tissue as soon as five minutes following unilateral nephrectomy [207].

Protein kinases

The EGF-receptor has a basic tyrosine kinase domain related to the p60 src protein [208, 209]. Similar tyrosine kinase domains can be found in the receptors for PDGF, IGF I, insulin, CSF-I as well as in receptor-like proteins encoded by the oncogenes c-neu, c-ros, c-mes and c-kit. In addition, tyrosine kinases exist which lack a transmembrane domain, and are, therefore, linked to the membrane via myristylated glycine [210]. These proteins can be formally considered as transducers. Recently, a new group of tyrosine kinases with a transmembrane domain, but without an external ligand binding domain have been described [211]. The further generation of multiple kinases is achieved by alternative splicing of single genes [212]. Speculations have been made that as much as a thousand different kinases are encoded by the mammalian genome [213]. All of these proteins are probably modifying signal transduction through interference with receptors and/or G-proteins. C-terminal lysine residues of the tyrosine kinase might also be involved in nucleotide binding since deletion of lysine inactivates the enzyme. The further role of tyrosine kinase in signal transduction is not clear. Tyrosine kinases can be involved in receptor internalization. In PDGF-stimulated 3T3 cells, an 85,000 Mr phosphoprotein, was identified one minute after treatment, suggesting this protein can be a substrate for the tyrosine kinase of the PDGF receptor [211]. Furthermore, this 85,000 Mr phosphoprotein might map with phosphatidylinositol (PI) kinase activity. The protein kinases encoded by the oncogenes c-src and c-ros are also associated with PI kinase activity [157, 159]. In EGF-stimulated cells, a 40 S ribosomal subunit protein called S6, with a molecular weight of 33,000 Mr, is immediately phosphorylated [214–216]. This 33 to 35,000 Mr protein is detectable in medullary and cortical thick ascending limbs, but not in proximal tubules in the rat kidney [217]. EGF-induced a threefold increase in S6 kinase phosphorylation after 30 minutes. Moreover, S6 phosphorylation increased in vivo four hours after uninephrectomy [218]. It has been hypothesized that the phosphorylation of S6 alters the affinity of the 40 S subunit for mRNA. This notion suggests the possibility of gene regulation at a transcriptional level [215]. The increased protein product might then perform by itself as a transacting factor in the subsequent regulation of other genes. Binding of insulin to its receptor also leads to the autophosphorylation of at least five tyrosine residues in the β-subunit of the receptor [219]. Autophosphorylation of the receptor is necessary for the further phosphorylation of endogenous substrates. Similar mechanisms of autophosphorylation upon signal stimulation have been described for PDGF, IGF-I, and EGF receptors. Additional complexity is added by the fact that different phosphoprotein phosphatases might be involved in the regulation of protein phosphorylation [220].

The strict separation of the various signal transduction pathways, however, is somewhat artificial, and a lot of networking between systems probably occurs [221]. PKC activation by phorbols ester, for example, increases cAMP in the presences of cholera toxin [222]. Vasopressin, in addition, which activates PKC but normally not adenylate cyclase, also enhances cAMP in 3T3 cells treated with forskolin or cholera toxin [223]. On the other hand, treatment of certain tissues with dibutyryl cAMP or theophyline can block phosphoinositide breakdown. Phosphorylation of phospholipase C by cAMP dependent kinases has been offered as the explanation. In addition to the above-mentioned autophosphorylation of tyrosine in the EGF receptor, serine and threonine residues are phosphorylated after EGF stimulated PKC. A 32,000 Mr myristaylated PKC substrate with a binding site for calmodulin has been described [224]. In one proposed model, phosphorylation of this protein leads to a release of bound calmodulin which, after Ca^{++}-binding, can activate other enzymes linking PKC to Ca^{++} activated processes [224]. The above-mentioned examples propose that interactive cross-talk between different transmembrane signalling systems can be involved in the regulation of renal enlargement.

Cellular pH and Na^+/H^+-antiporters

An enormous amount of work suggests that the Na^+/H^+-antiporter is involved in mitogenesis [225, 226] because alkalinization has been traditionally considered a prerequisite for cell proliferation. Several lines of evidence suggest the involvement of Na^+/H^+ exchange in this process: in controlled systems, proliferation can be induced by alkalinization in the absence of mitogens, also, most mitogenic substances stimulate Na^+/H^+ exchange (at least to some extent), inhibition of the Na^+/H^+-antiporter can hinder cellular enlargement, and enlargement is impaired in mutant cells lacking the antiporter [227, 228]. PKC, through stimulation by growth factors or directly by phorbol ester, can likewise activate the antiporter. Other kinases may also activate the antiporter. The molecular mechanisms surrounding those events are not entirely clear.

Activation of the Na^+/H^+ antiporter by growth factors in 3T3 fibroblasts is blocked by calcium antagonists and calmodulin inhibitors, indicating involvement of calmodulin-dependent transduction events. Direct phosphorylation of the antiporter has also been proposed, since activation is reduced in ATP-depleted cells and exchanger activity is increased in the presence of phosphatase inhibitors. Luminal Na^+/H^+ exchanger activity is decreased in proximal tubules with cAMP analogues, implying that stimuli which increase cAMP provide a negative regulation element for the antiporter [229]. The Na^+/H^+ exchanger in apical membranes of the proximal tubule is under dual phosphorylation control: protein phosphorylation mediated by PKC stimulates the exchanger whereas cAMP-dependent protein kinases inhibit activity by phosphorylation [230]. The early activation of the Na^+/H^+ exchanger is unaffected by actinomycin D making regulation through *de novo* protein synthesis unlikely. An increase in Na^+/H^+ antiporter activity in compensatory renal enlargement has been reported in several species as early as within 15 minutes after uninephrectomy [207]. Insulin and hydrocortisone in BSC-1 cells increase Na^+/H^+ antiporter activity as well as induce proliferation [58]. By adding TGFβ this stimulation can be converted into a hypertrophic response without proliferation. Antiporter activity, however, remains stimulated under these new conditions. Thyroid hormone increases Na^+/H^+ exchange in renal proximal tubule membrane vesicles, offering one explanation for how thyroid hormones can modify renal enlargement [231]. In isolated perfused proximal tubules neither insulin nor IGF-I could induce intracellular alkalinization of cells, whereas IGF-II would [232]. The IGF-II alkalinization was associated with an activated Na^+/H^+ exchanger. Very recently, preliminary results suggest that the ipsolateral denervation associated with unilateral nephrectomy might be a stimulus to the activation of the antiporter in the contralateral remnant kidney. This increased antiporter activity was thought to be mediated by contralateral renal innervation through alpha 1 receptors [233]. Activation of the renal tubular antiporter seems, therefore, to be a common event in both mitogenic and hypertrophic responses in the kidney.

More recently the general finding of Na^+/H^+ antiporter activity in renal enlargement has received increasing scrutiny [234, 235], especially since more information has accumulated indicating that amiloride has a variety of direct effects on protein synthesis independent from the inhibition of the Na^+/H^+ antiporter. Amiloride directly blocks reticulocyte lysate protein synthesis, interferes with DNA replication, influences topoisomerase II activity, and inhibits other transport systems important for enlargement, like the Na^+-linked cotransport of amino acids [236]. In accordance with this view, amiloride, but not hexamethylene amiloride, which has a much higher affinity for the Na^+/H^+-antiporter, was shown to reduce the compensatory increase in kidney weight and protein content after uninephrectomy in mice. These findings indicate an inhibition of protein synthesis by amiloride unrelated to antiporter activity [237]. Mutant kidney pig epithelial cells (LLC-PK1) and hamster CCL39 fibroblasts deficient of Na^+/H^+ antiporter can grow in medium containing bicarbonate, or at alkaline pH, indicating that pH, and not the activation of the antiporter, is essential for cell enlargement. Proliferation can be induced in LLC-PK1 cells in the absence of Na^+/H^+ exchange activity [238]. IGF-I or insulin-induced hypertrophy was not different between LLC-PK1 mutants and wild type cells, suggesting that antiporter activity is not necessary, either, for hypertrophy in this model [239]. Taken together, these findings suggest that activation of Na^+/H^+ exchange is not a necessary prerequisite for the enlargement of tubular cells. The exchanger might rather be activated in parallel with other molecular mechanisms having a more central role in the pluripotential growth process.

Immediate early genes

Stimulation of rested cells with serum or growth factors like PDGF, FGF, or EGF induces, after one hour, the expression of cellular oncogenes c-fos, c-jun and c-myc [240]. The activation of c-jun A and c-fos precedes the induction of c-myc [241, 242]. After the addition of PDGF to quiescent fibroblasts, a 50-fold increase in c-fos expression has been observed within 30 minutes, whereas c-myc RNA reaches a peak level after one to two hours. More recently, a variety of other early genes, induced by growth factors with similar kinetics to c-fos, have been described [243, 244], including Krox-20, KC, JE, fos B, Fra-1, E1A, c-myb, EGR and jun B. More than 70 different genes induced by serum have been identified by differential screening of cDNA libraries prepared from serum-stimulated 3T3 cells [245]. In terms of induction kinetics and RNA half-lives, two groups of immediate early genes can be distinguished: one group represented by c-fos, are necessary for the transition from the G_0 and the G_1-phase of the cell cycle, but are not required for further progression. In contrast, genes like c-myc are detectable at basal levels through all stages of the cell cycle. These genes might be necessary for the transition from G_1 to the S phase. A common finding of immediate early genes is their superinduction in the presence of the protein inhibitor cycloheximide [246]. Superinduction after cycloheximide treatment may be due to the inhibition of transcriptional blockers and the enhancement of RNA stability by restricting *de novo* synthesis of RNA degrading enzymes.

c-fos and c-jun

The c-fos gene has four coding exons resulting in a mRNA of 2.2 kb [247]. The fos promoter, localized upstream of the AUG start site, is under complex positive and negative regulation [248, 249]. C-fos protein, itself, has auto-negative feedback transregulatory activity on its promoter [250]. The c-fos promoter respond to agents which activate intracellular PKC or

cAMP probably because it contains cAMP response elements [251]. The c-fos protein forms a heterodimer with the protein product of c-jun [252]. Association of the both proteins occurs through a leucine zipper domain [253]. Dimerization is necessary for subsequent DNA binding, but binding of the heterodimer to the specific DNA sequence TGACTCA present in a variety of enhancers and promoters occurs via a domain distinct from the leucine zipper [254]. While fos has an inherent DNA-binding activity of its own, it still cannot alone form homodimeric structures necessary for binding. Although site-directed mutagenesis experiments demonstrated that the regions in fos and jun responsible for DNA binding are conserved, suggesting that fos and jun interact each with one-half site of the target sequence [255, 256], recent evidence now reveals that the binding is asymmetrical [257]. Protein products from newer oncogenes related to fos or jun like fos B and jun B might form leucine zipper in a similar fashion, or gene products of jun/fos families might be even interchangeable in building the dimer [258]. As already mentioned above, c-jun is a member of a larger family, including jun B and jun D. All members share a leucine zipper motif as well as DNA binding domains [258]. Similar to c-fos, c-jun is inducible by serum and growth factors [259]. In contrast to fos, c-jun, however, can be identified at low levels in uninduced cells, and is positively transautoregulated by its product [260, 261]. This positive autoregulation could be responsible for prolonging and extending transduction signals. Thus, c-fos and c-jun are both independently induced by different signal transduction systems after the stimulation of cells with growth factors. Both genes are under control of complex enhancer/promoter regulation. Their products subsequently form heterodimers through leucine zippers, and then bind to specific sequences in enhancer/promoter regions of the target genes. Other products of this family of genes might also be involved in this process, or compete one with another for dimerization [262]. It is obvious that such a complicated network offers the opportunity of regulatory modulation at different levels.

c-myc

The murine c-myc has three exons of which the first exon contains non-coding regulatory functions in mice, but has an open reading frame in humans [263]. Transcription of the gene initiates from two promoters named P1 and P2 which are independently regulated and associated with a TATA box [264]. Regulation of c-myc occurs at transcriptional and post-transcriptional levels. The c-myc gene is subject to negative and positive regulation involving cis- and transacting factors [265, 266]. A variety of binding sites for activators and repressors have been described in the large conserved c-myc promoter region, in the 5′ flanking region of the gene and in the first intron [267]. Transcriptional elongation is negatively regulated by an element inducing an intragenic pause in the first exon. It has been postulated that different growth factors might regulate c-myc transcription by several mechanisms [266]. PKC-dependent pathways, for example, may activate transcription, whereas cAMP-dependent pathways reduce intragenic pausing [267]. This distinction, however, seems to be artificial since different gene regulatory factors might not act independently. The c-myc protein has a molecular weight of 62 to 64,000 Mr, is phosphorylated, and has a half-life of 15 minutes. This c-myc protein, when micro-injected, can induce DNA synthesis in quiescent 3T3 cells [157].

Immediate early gene expression in renal growth

A 30-fold increase in the expression of c-myc transcripts has been reported in kidneys of homozygous recessive C57BL/6J mice which develop massively cystic nephrons. Interestingly enough, the expression of S-phase-associated H4 RNA increases only modestly, suggesting that elevated c-myc expression is not solely a marker for proliferation [268]. Mice positive for c-myc transgene driven by an SV40 enhancer also develop polycystic kidney disease, and die of renal failure between three weeks and five months of age [269]. The proliferation of tubular cells, subsequent to acute injury induced by folic acid treatment, is associated with an increase in oncogene expression [270, 271]. S1 nuclease mapping demonstrates c-myc transcripts which originate from the P1 and P2 initiation sites (with a biased use of the P2 site) within two hours of folic acid treatment. Nuclear run-off assays, however, reveal evidence for a parallel increase in the blocking of transcriptional elongation. In contrast, c-fos transcription is induced after folic acid treatment without affecting elongation block mechanisms. The authors conclude from their study that the increase in c-myc expression, in contrast to c-fos, was probably due to post-transcriptional mechanisms, since the amount of effective transcription of c-myc remains constant despite the increase of c-myc transcripts from the P1 and P2 sites [270]. A tenfold increase of c-myc expression in renal cortex is observed during the repair phase of ischemic acute renal failure [272]. Similar changes have been detected after renal ischemia for c-fos and the immediate early gene, Egr-1, which shares induction kinetics with c-fos [87]. The mitogens ADP, EGF, and PDGF stimulate c-fos and c-myc expression in different tubular epithelial cell lines.

Very modest increases in c-myc, c-H-ras and c-K-ras expression have been reported in compensatory renal hypertrophy after uninephrectomy [273, 274]. Such observations, however, may be related to operative stress, since oncogene expression also increases in sham-operated animals. A more sustained expression of c-H-ras and c-K-ras after unilateral nephrectomy, however, has been described [275]. Although the in vivo studies using total kidney RNA after uninephrectomy suggest that oncogene expression does not change, cell-type specific changes might exist which can only be addressed by the more sensitive method of in situ hybridization. Moreover, the non-specific increase in oncogene expression associated with surgery and anesthesia may veil a slight, but biologically significant, rise in immediate early gene expression after nephrectomy. Recently, Sawczuk and co-workers found an transitory induction of c-fos and c-myc in contralateral kidneys 15 minutes after acute unilateral ureteral obstruction [276]. In this model, acute unilateral obstruction led to a hypertrophic compensatory enlargement response in the contralateral kidney [277]. Oncogene expression was also observed in the obstructed kidney associated with tubular necrosis and stromal proliferation. These results suggest that oncogene induction, after unilateral ureteral obstruction, is associated with hypertrophy (contralateral kidney) as well as with proliferation (ipsolateral kidney). In a more recent study, Egr-1 expression was elevated 30 minutes after contralateral nephrectomy when compared with sham-operated

control animals [278]. These collective findings seem to indicate that expression of some immediate early genes might be a feature common to hypertrophic and proliferative responses in tubular cells.

Regulation of renal growth by steroid hormones and retinoic acid

Although androgens are not a prerequisite for compensatory renal hypertrophy, these hormones might modulate the enlargement response of tubular cells [279]. Treatment of female mice, but not of male mice, with exogeneous testosterone induces hypertrophy in proximal tubules, whereas other organs, like the liver, demonstrate a more combined effect of hypertrophy and hyperplasia, suggesting tissue-specific enlargement effects of androgens [280]. Impairment of compensatory renal hypertrophy in hypothyroidism has been described suggesting modification of renal enlargement by thyroid hormones [281]. Although thyroid hormones have other actions like stimulation of the Na^+/H^+ antiporter or induction of EGF-receptors (see above), their main regulatory function is probably most visible directly at the gene level [282]. Steroid hormones bind to special steroid receptors. Structurally similar receptors exist for thyroid hormones [283]. The steroid-steroid receptor complex subsequently binds to a special hormone response element localized in promoter/enhancer regions of many genes [284]. Receptors for different steroids are structurally related, and each receptor can be divided into six regions (A-F) depending on the degree of amino acid sequence homology [285–287]. The highly conserved C region contains the DNA binding domain, and is 84 amino acids long in the androgen receptor. The N-terminal A-B regions are variable in size between different receptors, whereas the C-terminal E-F regions of the receptor bind the appropriate hormone. Steroid-receptors bind in the absence of hormone to a 90,000 Mr heat-shock protein which masks its DNA-binding site. Steroid hormones promote dissociation of this complex. The DNA-binding C-region of the receptor consist of two zinc-finger motifs where each zinc atom is surrounded by four cysteines [288]. Target gene specificity is determined by these zinc-fingers. A model has been proposed in which a dimer of receptors bind to the major grooves of four helix turns of DNA [289]. Glucocorticoid response element, with the conserved hexanucleotide sequence TGTTCT, can also function as androgen response elements [290]. In addition, hormone response elements have been described which are unique to androgen-regulated genes [291].

A variety of genes and their gene products are induced in proximal tubule kidney cells of mice treated with androgens. Among them are enzymes like β-glucuronidase, ornithine decarboxylase, and alcohol dehydrogenase [292]. Furthermore, a 43,000 Mr protein with unknown function encoded by the RP2 gene is induced in the kidney through testosterone treatment [293]. In a similar manner, a polypeptide named kidney androgen-regulated protein (KAP) is induced tenfold in proximal convoluted tubular cells by androgen treatment. In addition to transcriptional regulation, there is increasing evidence that androgen inducible mRNA in kidney might be post-transcriptionally modified in some way [280, 293]. It is, however, unknown whether these post-transcriptional effects of steroids are mediated by direct interaction of steroid-receptors and RNA, resulting in stabilization of mRNA, or rather are indirect through changes in transcription rates of other genes which might, in turn, regulate the post-transcriptional event.

Retinoic acid potentiates the activity of EGF in rat kidney fibroblasts probably by increasing the number of EGF-receptors [294, 295]. Retinoic acid induces mitogenesis in rabbit kidney epithelial cells, whereas retinol was without any effect on proliferation [296]. Retinoic acid increased the size of domes in confluent Madin-Darby canine kidney cells [297]. The dome formation was associated with an increase in cAMP. Previous experiments have demonstrated that retinoic acid stimulates cAMP-dependent protein kinases A. Retinoic acid binding-protein, which is identical or homologous to the cellular receptors for retinoic acid (CRA), has been demonstrated in the urine of living-related kidney donors after uninephrectomy [298]. Furthermore, serum levels of vitamin A and retinol binding-protein are increased after uninephrectomy. Since CRAs are normally present in tubular cells, but do not appear in the urine, detection of this protein in the urine after uninephrectomy might reflect an increase in synthesis with spill over in the urine [298]. CRAs are members of the steroid receptor superfamily [283]. Alpha- and beta-receptors for retinoic acid have also been described. The receptor interacts with a hormone response element in target genes similar to other receptors of the steroid superfamily. Retinoic acid induces enlargement hormone gene expression, likewise suggesting the existence of HREs for the retinoic acid receptors in the flanking region of the growth hormone gene [299]. The alpha form of CRA can interact with the beta form of the thyroid hormone receptor on thyroid hormone response elements [300]. The retinoic acid-thyroid hormone receptor heterodimer binds to different target DNA sequences than the thyroid hormone receptor alone. It is tempting to speculate that heterodimer formation might occur between other members of the steroid receptor superfamily, explaining the complexity of transcriptional control by these hormones. The analogy of these mechanisms to the above-described jun-fos interaction is obvious.

Gene expression and regulation of transcription in renal growth

Kidney mRNA is polyadenylated in a heterogeneous fashion [301]. Around 50% of newly synthesized mRNA from kidneys lack or have only very short poly(A) tails. No changes in size, half-life, and distribution of total mRNA could be detected in compensatory renal enlargement. The percentage of *de novo* synthesized mRNA lacking polyadenylation, however, increased within 60% minutes after uninephrectomy [301, 302]. Since poly(A) containing and poly(A)-deficient mRNAs are homologous [303], it unclear whether these findings are involved in regulatory mechanisms associated with compensatory renal enlargement. Miller and McCarthy prepared radioactive labelled cDNA from kidney after uninephrectomy and hybridized the cDNA to mRNA prepared from normal kidneys [304]. The maximal hybridization was unchanged compared to a hybridization pattern from control cDNA to control mRNA. The authors concluded that only few, if any, new species of mRNA were expressed in compensatory renal hypertrophy. However, these early applied hybridization techniques were rather insensitive and could not measure small changes in mRNA expression. Moreover, the overall hybridization pattern, without actual cloning of the appropriate genes, could not

detect small changes in rates of transcription or expression of rare genes. Ouellette and co-workers, using nuclear run-off assays, demonstrated an increase in transcription of precursor rRNA 24 hours after uninephrectomy, whereas mRNA levels coding for ribosomal proteins were unchanged [305]. There is evidence, especially in androgen-induced hypertrophy, that mRNA stabilization on a post-transcriptional level is important [279]. It is, however, likely that mechanisms regulating post-transcriptional events are themselves under transcriptional control [306]. Although proliferative growth is characterized by *de novo* expression of different genes, including oncogenes, it is not really clear whether new transcription of distinct genes occurs during hypertrophy. No genes have been hitherto described which are only associated with hypertrophy, but not with mitogenesis. We believe that special genes likely exist which are activated or deactivated only in the setting of hypertrophy, and not in mitogenesis, since renal hypertrophy is a highly complex enlargement response involving several regulatory factors clearly distinct from hyperplasia. In addition, new genes which are specifically expressed at growth arrest, and are down regulated after induction of mitogenesis, have been recently described [307]. These genes, interestingly enough, were not expressed at equal levels in all tissues of the mouse. Recently a gene has been described which is only active in normal liver tissues but not during regenerating growth of the liver [308].

Early biochemical changes in renal growth

One of the earliest biochemical events in compensatory hypertrophy is an increased of choline in phospholipids. It is already detectable minutes after uninephrectomy [8, 309]. In parallel, the activity of the key enzymes, choline kinase and cholinephosphotransferase, are increased during the biosynthesis of phoshophatidylcholine [310]. An increase, furthermore, in phosphatidylcholine synthesis via the Kennedy pathway occurs in hyperplasic forms of renal enlargement, like acute tubular necrosis, or renal enlargement induced by potassium depletion [311]. An increase in the synthesis of phospholipids may reflect new membrane formation in growing kidney cells or modified expression of membrane-bound enzyme activity [8]. The mechanism of how growth factors stimulate phosphatidylcholine synthesis is not known. However, *de novo* synthesis of key enzymes and/or alterations in the membrane microenvironment with activation of membrane-associated enzymes may be important after binding of growth factors to cellular receptors. An increase of the enzyme ornithine decarboxylase (ODC), catalyzing the first step in polyamine biosynthesis, has been described after unilateral nephrectomy [15]. Pretreatment of rats with difluormethylornithine, an inhibitor of ODC, did not influence renal hypertrophy after ablation, suggesting that this enzyme is not directly involved in the mechanisms of compensatory renal hypertrophy [312]. The activities of various enzymes of the proximal tubule, including Na^+-K^+-ATPase [313, 314], aminopeptidases [315], and the enzymes of gluconeogenesis [316], are increased in compensatory enlargement reflecting final biochemical alterations which might lead to a form of hypermetabolism [317], and contribute to the long-term progression of chronic renal failure in remnant kidneys. Prostaglandins are likewise involved in the regulation of renal enlargement. Reduction in renal mass stimulates the synthesis of prostaglandins [318, 319]. Furthermore, eicosanoids might be responsible, in part, for the increase in renal blood flow and glomerular filtration occurring after ablation of renal mass. Treatment of uninephrectomized rats with indomethacin likewise abolished the increase in prostaglandin synthesis in compensatory renal enlargement [320]. Growth factor activation of phospholipase C and phospholipase A_2 also releases arachidonic acid from membrane phospholipids and stimulates prostaglandin synthesis [182]. Purkerson et al reported increased thromboxane B_2 urinary excretion in rats with 1-1/3 nephrectomy [321]. Treatment with OKY 1581, an inhibitor of thromboxane synthesis, resulted in better renal function and less severe histological changes, but no effect on kidney size was observed, indicating that, at least in this setting, thromboxanes are not directly involved in compensatory renal enlargement.

Summary

Adult kidneys, which are principally composed of tubulointerstitium, do not normally regenerate or expand their working pool of functional cells at a very high rate. Loss of kidney tissue, however, can lead to some compensatory renal enlargement. The catalytic forces initiating such exchanges have not been fully articulated by current experimental endeavors. Increasing evidence, nevertheless, does suggest that factors other than simple changes in renal hemodynamics may be involved in this process. Different cellular elements in the tubulointerstitial microenvironment probably modulate changes in tubular enlargement or size through a complex cytokine network. Autocrine and paracrine stimulation of enlargement by different local growth factors also seem to play a pivotal role. After binding to cellular receptors, these factors activate signal transduction pathways resulting in expression of immediate early genes, which by themselves can synchronize the expression of subsequent genes through the medium of transacting factors. The renal enlargement response can also be modified by endocrine hormones that can activate such genes directly and/or stimulate other adjunctive processes, like receptor expression for the regional binding of growth factors. Furthermore, renal enlargement is under negative feedback of inhibitory factors like TGFβ. It is possible, for example, that special genes exist which are only expressed to arrest enlargement. It has been further suggested that activation of the Na^+/H^+ antiporter is a common denominator in renal enlargement. Recent findings, however, indicate that the activation of this antiporter is not always necessary, and might rather be a parallel event rather than a key phenomena in tubular enlargement. G_0/G_1 transition of tubular cells seems to involve similar factors in tubular hypertrophy and hyperplasia. The factors which are responsible for the final determination of the enlargement pattern (hypertrophy vs. proliferation) are unknown. The separation between hypertrophy and hyperplasia, although suggested by striking differences in cellular regulation, may be somewhat artificial, since responses leading to tubular enlargement also exist in circumstances where hyperplasia and hypertrophy are combined events. Recently it has been proposed that growth factors stimulate gluconeogenesis in proximal tubular cells producing hyperplasia, whereas factors inhibiting gluconeogenesis might induce hypertrophy [81]. Whether the common pathway message of this intriguing hypothesis is correct still requires further validation. The subsequent activation of structural genes, in-

cluding collagen secretion, membrane enzymes, tubular hypermetabolism and induced immune mechanisms, are also important. These latter factors tend to modify the development of structural diversity and interstitial fibrosis over the longer time frame of the compensatory responses producing enlargement. The regulatory mechanisms of tubular enlargement are fascinatingly complex, and certainly many more informative molecular processes will be described in the future.

Acknowledgments

Dr. Wolf is a recipient of a fellowship from the Boehringer Ingelheim Funds, Stuttgart, FRG. Dr. Neilson is the recipient of an Established Investigator Award (85-108) from the American Heart Association and its Pennsylvania affiliates. Original work in the authors laboratory was supported by grants from National Institute of Health (DK-30280, DK-41110, DK-07006, and AR-25530).

Reprint requests to Eric G. Neilson, M.D., Renal-Electrolyte Section, 700 Clinical Research Building, University of Pennsylvania, 422 Curie Boulevard, Philadelphia, Pennsylvania 19104-6144, USA.

References

1. Fine L: The biology of renal hypertrophy. *Kidney Int* 29:619–634, 1986
2. Hostetter TH, Olson JL, Rennke HG, Venkatachlam MA, Brenner BM: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. *Am J Physiol* 241:F85–F93, 1981
3. Brenner BM: Nephron adaptation to renal injury or ablation. *Am J Physiol* 249:F324–F337, 1985
4. Katz AI, Toback FG, Lindheimer MD: The role of renal work in compensatory kidney growth. *Yale J Biol Med* 51:331–337, 1978
5. Yoshida Y, Fogo A, Ichikawa I: Glomerular hemodynamic changes versus hypertrophy in experimental glomerular sclerosis. *Kidney Int* 35:654–660, 1989
6. Fogo A, Yoshida Y, Glick AD, Homma T, Ichikawa I: Serial micropuncture analysis of glomerular function in two rat models of glomerular sclerosis. *J Clin Invest* 82:322–330, 1988
7. Scherberich JE, Wolf G, Albers C, Nowack A, Stuckhardt C, Schoeppe W: Glomerular and tubular membrane antigens reflecting cellular adaptation in human renal failure. *Kidney Int* 36 (Suppl. 27):S38–S51, 1989
8. Toback FG: Phosphatidylcholine metabolism during renal growth and regeneration. *Am J Physiol* 246:F249–F259, 1984
9. Katz AI, Toback FG, Lindheimer MD: Independence of onset of compensatory kidney growth from changes in renal function. *Am J Physiol* 230:1067–1071, 1976
10. Hirschberg R, Kopple JD: Evidence that insulin growth factor I increases renal plasma flow and glomerular filtration rate in fasted rats. *J Clin Invest* 83:326–330, 1989
11. Harris RC, Hoover RL, Jacobson HR, Badr KF: Evidence for glomerular actions of epidermal growth factor in the rat. *J Clin Invest* 82:1028–1039, 1988
12. Johnson HA, Vera Roman JM: Compensatory renal enlargement: Hypertrophy versus hyperplasia. *Am J Physiol* 49:1–13, 1966
13. Fine LG, Bradley T: Adaptation of proximal tubular structure and function: Insights into compensatory renal hypertrophy. *Fed Proc* 44:2723–2727, 1985
14. Wesson LG: Compensatory growth and other growth responses of the kidney. *Nephron* 51:149–184, 1989
15. Malt RA: Compensatory growth of the kidneys. *N Engl J Med* 280:1446–1459, 1969
16. Austin HA, Goldin H, Gaydos D, Preuss HG: Polyamine metabolism in compensatory renal growth. *Kidney Int* 23:581–587, 1983
17. Lewin B: Driving the cell cycle: M phase kinase, its partners, and substrates. *Cell* 61:743–753, 1990
18. Murray AW, Kirschner MW: Dominoes and clocks: The union of two views of the cell cycle. *Science* 246:614–621, 1989
19. McIntosh JR, Koonce MP: Mitosis. *Science* 246:622–628, 1989
20. Pardee AB: G_1 events and regulation of cell proliferation. *Science* 246:603–608, 1989
21. Kaczmarek L: Protooncogene expression during the cell cycle. *Lab Invest* 54:365–376, 1986
22. Fine LG, Norman J: Cellular events in renal hypertrophy. *Ann Rev Physiol* 51:19–32, 1989
23. Sunkara PS, Wright DA, Rao PN: Mitotic factors from mammalian cells induce germinal vesicle breakdown and chromosome condensation in amphibian oocytes. *Proc Natl Acad Sci USA* 76:2799–2802, 1979
24. Cross F, Roberts J, Weintraub H: Simple and complex cell cycles. *Ann Rev Cell Biol* 5:341–395, 1989
25. Gautier J, Norbury C, Lohka M, Nurse P, Maller J: Purified maturation-promoting factor contains the product of a Xenopus homolog of the fission yeast cell cycle control gene $cdc2^+$. *Cell* 54:433–439, 1988
26. Moria AO, Draetta G, Beach D, Wang JYJ: Reversible tyrosine phosphorylation of cdc2: Dephosphorylation accompanies activation during entry in mitosis. *Cell* 58:193–203, 1989
27. Draetta G, Brizuela L, Potashkin J, Beach D: Identification of p 34 and p 13, human homologs of the cell cycle regulators of fission yeast encoded by $cdc2^+$ and $suc1^+$. *Cell* 50:319–325, 1987
28. Arion D, Meijer L, Brizuela L, Beach D: Cdc2 is a component of the M phase-specific histone H1 kinase: Evidence for identity with MPF. *Cell* 55:371–378, 1988
29. Murray AW: Cyclins in meiosis and mitosis. *Nature* 326:542–543, 1987
30. Draetta G, Luca F, Westendorf J, Brizuela L, Ruderman J, Beach J: Cdc2 protein kinase is complexed with both cyclin A and B: Evidence for proteolytic inactivation of MPF. *Cell* 56:829–838, 1989
31. Cisek LJ, Corden JL: Phosphorylation of RNA polymerase by the murine homologue of the cell-cycle control protein cdc2. *Nature* 339:679–684, 1989
32. Gould KL, Nurse P: Tyrosine phosphorylation of the fission yeast $cdc2^+$ protein kinase regulates entry into mitosis. *Nature* 342:39–45, 1989
33. Russell P, Nurse P: $Cdc25^+$ functions as an inducer in the mitotic control of fission yeast. *Cell* 45:145–153, 1986
34. Russell P, Nurse P: Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog. *Cell* 49:559–567, 1987
35. Adlakha RC, Sahasrabuddhe CG, Wright DA, Rao PN: Evidence for the presence of inhibitors of mitotic factors during G_1 period in mammalian cells. *J Cell Biol* 97:1707–1713, 1983
36. Murray AW: The cell cycle as a cdc2 cycle. *Nature* 342:14–15, 1989
37. Tisher CC, Madsen KM: Anatomy of the renal interstitium, in *Nephrology* (vol. I), Proc. Xth Int. Congr. Nephrology, London, Bailliere Tindall, 1988, pp. 587–598
38. Neilson EG: Pathogenesis and therapy of interstitial nephritis. *Kidney Int* 35:1257–1270, 1989
39. Haverty T, Kelly CJ, Neilson EG: Immune-mediated renal disease. *Curr Nephrol* 10:1–36, 1987
40. Alvarez RJ, Haverty T, Neilson EG: Functional heterogeneity among anatomically distinct fibroblasts: Implications for immune regulation of function (abstract). *Kidney Int* 35:308, 1989
41. Alvarez RJ, Haverty T, Neilson EG: Analysis of functional differences in the growth and synthesis of tubulointerstitial fibroblast and tubular epithelium (abstract). *Kidney Int* 33:149, 1988
42. Norman JT, Kleinman KS, Bacay A, Fine LG: Renal tubular epithelial cells release factors which modulate fibroblast function: Implications for pathogenesis of interstitial nephretis. (abstract) *Kidney Int* 35:179, 1989
43. Neilson EG, Jimenez SA, Phillips SM: Cell-mediated immunity in interstitial nephritis. III. T lymphocyte-mediated fibroblast proliferation and collagen synthesis: An immune mechanism for renal fibrogenesis. *J Immunol* 125:1708–1714, 1980
44. Nathan CF: Secretory products of macrophages. *J Clin Invest* 79:319–326, 1987
45. Freundlich B, Bomalaski JS, Neilson EG, Jimenez SA:

Immune regulation of fibroblast proliferation and collagen synthesis by soluble factors from mononuclear cells. *Immunol Today* 7:303–307, 1986
46. COTRAN RS, POBER JS: Effects of cytokines on vascular endothelium: Their role in vascular and immune response. *Kidney Int* 35:969–975, 1989
47. COTRAN RS: New roles for the endothelium in inflammation and immunity. *Am J Pathol* 129:407–413, 1987
48. DICORLETO PE, BOWEN-POPE DF: Cultured endothelial cells produce a platelet-derived growth factor-like protein. *Proc Natl Acad Sci USA* 80:1919–1923, 1983
49. BRAUN-MENENDEZ E: Evidence for renotropin as a causal factor in renal hypertension. *Circulation* 17:696–701, 1958
50. MANZANO F, ESBRIT P, GARCIA-OCANA A, GARCIA-CANERO R, JIMENEZ-CLAVERO MA: Partial purification and characterisation of a renal growth factor from plasma of uninephrectomised rats. *Nephrol Dial Trans* 4:334–338, 1989
51. MENDLEY SR, TOBACK FG: Autocrine and paracrine regulation of kidney epithelial growth. *Ann Rev Physiol* 51:33–50, 1988
52. KUJUBU DA, FINE LG: Physiology and cell biology update: Polypeptide growth factors and their relation to renal disease. *Am J Kidney Dis* 14:61–73, 1989
53. PATT LM, HOUCK JC: Role of polypeptide growth factors in normal and abnormal growth. *Kidney Int* 23:603–610, 1983
54. DEUEL TF: Polypeptide growth factors. Roles in normal and abnormal cell growth. *Ann Rev Cell Biol* 3:443–492, 1987
55. BURCK KB, LIU ET, LARRICK JW: Growth factors and receptors, in *Oncogenes*, New York, Springer, 1988, pp. 156–181
56. HORSTER MF, STOPP M: Transport and metabolic functions in cultured renal tubule cells. *Kidney Int* 29:46–53, 1986
57. HAVERTY TP, KELLY CJ, HINES WH, AMENTA PS, WATANABE M, HARPER RA, KEFALIDES NA, NEILSON EG: Characterization of a renal tubular epithelial cell line which secretes the autologous target antigen of autoimmune experimental interstitial nephritis. *J Cell Biol* 107:1359–1368, 1988
58. FINE LG, HOLLEY RW, NASRI H, BADIE-DEZFOOLY B: BSC-1 growth inhibitor transforms a mitogenic stimulus into a hypertrophic stimulus for renal proximal tubular cells: Relationship to Na^+-H^+ antiport activity. *Proc Natl Acad Sci USA* 82:6163–6166, 1985
59. FINE LG, BADIE-DEZFOOLY B, LOWE AG, HAMZEH A, WELLS J, SALEHMOGHADDAM S: Stimulation of Na^+/H^+ antiport is an early event in hypertrophy of renal proximal tubule cells. *Proc Natl Acad Sci USA* 82:1736–1740, 1985
60. WOLF G, NEILSON EG: Angiotensin II induces cellular hypertrophy in a murine proximal tubular cell line. *Am J Physiol* 259:F768–F777, 1990
61. HELDIN CH, WESTERMARK B: PDGF-like growth factor in autocrine stimulation of growth. *J Cell Physiol* 5 (Suppl):31–24, 1987
62. BETSHOLZ C, JOHNSSON AN, HELDIN CH, WESTERMARK B, LIND P, URDEA MS, EDDY R, SHOWS TB, PHILPOTT K, MELLOR AL: cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumor cell lines. *Nature* 320:695–709, 1986
63. ROSS R, RAINES EW, BOWEN-POPE DF: The biology of platelet-derived growth factor. *Cell* 46:155–169, 1986
64. RAO CH, IGARASHI H, CHIU IM, ROBBINS KC, AARONSON SA: Structure and sequence of the human c-sis/platelet-derived growth factor 2 (SIS/PDGF 2) transcriptional unit. *Proc Natl Acad Sci USA* 83:2392–2396, 1986
65. STILES CD: The molecular biology of platelet-derived growth factor. *Cell* 33:653–655, 1983
66. HELDIN CH, WESTERMARK B: Platelet-derived growth factors: A family of isoforms that bind to two distinct receptors. *Br Med Bull* 45:438–452, 1989
67. KARTHA S, BRADHAM DM, GROTENDORST GR, TOBACK FG: Kidney epithelia cells express the c-sis proto-oncogene and secrete PDGF-like protein. *Am J Physiol* 255:F800–F806, 1988
68. COLLINS T, GINSBURG D, BOSS JM, ORKIN SH, POBER JS: Cultured human endothelial cells express platelet-derived growth factor B chain: cDNA cloning and structural analysis. *Nature* 316:748–750, 1985
69. CARPENTER G, ZENDEGUI JG: Epidermal growth factor, its receptor, and related proteins. *Exp Cell Res* 164:1–10, 1986
70. CARPENTER G, COHEN S: Epidermal growth factor. *Ann Rev Biochem* 48:193–216, 1979
71. GRAY A, DULL TJ, ULLRICH A: Nucleotide sequence of epidermal growth factor cDNA predicts a 128,000 molecular weight precursor. *Nature* 303:722–725, 1983
72. HIGUCHI Y, MARIMOTO Y, HARINATA A, YASUOKA N: Crystalization and preliminary X-ray studies of human epidermal growth factor. *J Biochem* 103:905–906, 1988
73. WATERFILED MD: Epidermal growth factor and related molecules. *Lancet* i:1243–1246, 1989
74. OLSEN PS, NEXO E, POULSEN SS, HANSEN HF, KIRKEGAARD P: Renal origin of rat urinary epidermal growth factor. *Reg Pept* 10:37–45, 1984
75. FISHER DA, SALIDO EC, BARAJAS L: Epidermal growth factor and the kidney. *Ann Rev Physiol* 51:67–80, 1989
76. SALIDO EC, YEN PH, SHAPIRO LJ, FISHER DA, BARAJAS L: In situ hybridisation of prepro-epidermal growth factor mRNA in the mouse kidney. *Am J Physiol* 256:F632–F638, 1989
77. LAU JLT, FOWLER JE, GHOSH L: Epidermal growth factor in the normal and neoplastic kidney and bladder. *J Urol* 139:170–175, 1988
78. SALIDO EC, BARAJAS L, LECHAGO J, LABORDE NP, FISHER D: Immunocytochemical localization of epidermal growth factor in mouse kidney. *J Histochem Cytochem* 34:1155–1160, 1986
79. SALIDO EC, FISCHER DA, BARAJAS L: Immunoelectron microscopy of epidermal growth factor in mouse kidney. *J Ultrastruct Mol Struct Res* 96:105–113, 1986
80. GOODYER PR, KACHRA Z, BELL C, ROZEN R: Renal tubular cells are potential targets for epidermal growth factor. *Am J Physiol* 255:1191–1196, 1988
81. HARRIS RC, DANIEL TO: Epidermal growth factor binding, stimulation of phosphorylation, and inhibition of gluconeogenesis in rat proximal tubule. *J Cell Physiol* 139:383–391, 1989
82. USHIRO H, COHEN S: Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cells. *J Biol Chem* 255:8363–8369, 1980
83. MUKKU VR: Regulation of epidermal growth factor receptor levels by thyroid hormones. *J Biol Chem* 259:6543–6547, 1984
84. JANSSON JO, EKBERG S, HOATH SB, BEAMER WG, FROHMAN LA: Growth hormone enhances hepatic epidermal growth factor receptor concentration in mice. *J Clin Invest* 82:1871–1876, 1988
85. ZOON KC, KARASAKI Y, ZUR NEDDEN DL, HU R, ARNHEITER H: Modulation of epidermal growth factor receptors by human α interferon. *Proc Natl Acad Sci USA* 83:8226–8230, 1986
86. NORMAN J, BADIE-DEZFOOLY B, NORD EP, KURTZ I, SCHLOSSER J, CHAUDHARI A, FINE LG: EGF-induced mitogenesis in proximal tubular cells: Potentiation by angiotensin II. *Am J Physiol* 253:F299–F309, 1987
87. SAFIRSTEIN R, PRICE PM, SAGGI SJ, HARRIS RC: Changes in gene expression after temporary renal ischemia. *Kidney Int* 37:1515–1521, 1990
88. HUMES HD, CIESLINSKI DA, COIMBRA TM, MESSANA JM, GALVAO C: Epidermal growth factor enhances renal tubule cell regeneration and repair and accelerates the recovery of renal function in postischemic acute renal failure. *J Clin Invest* 84:1757–1761, 1989
89. JENNISCHE E, ANDERSSON G, HANSSON HA: Epidermal growth factor is expressed by cells in the distal tubules during postnephrectomy renal growth. *Acta Physiol Scand* 129:449–450, 1987
90. SALIDO EC, LABORDE NP, BUENAFLOR G, GRODIN M, BARAJAS L, FISHER DA: Renal EGF after unilateral nephrectomy. (abstract) *Clin Res* 36:245A, 1988
91. SACK E, ARRUDA JAL, TALOR Z: EGF binding is decreased in compensatory renal hypertrophy. (abstract) *Kidney Int* 35:319, 1989
92. UCHIDA S, TSUTSUMI O, HISE MK, OKA T: Role of epidermal growth factor in compensatory renal hypertrophy in mice. (abstract) *Kidney Int* 33:387, 1988
93. BEHRENS MY, CORBIN AL, HISE MK: Epidermal growth factor receptor regulation in rat kidney: Two models of renal growth. *Am J Physiol* 257:F1059–F1064, 1989
94. JANSEN M, VAN SCHAIK FMA, RICKER AT, BULLOCK B, WOODS

DE, GABBAY KH, NUSSBAUM AL, SUSSENBACH JS, VAN DEN BRADE JL: Sequence of cDNA encoding human insulin-like growth factor I precursor. *Nature* 306:609–611, 1983
95. ZAPF Z, FROESCH ER: Insulin-like growth factors/somatomedins: Structure, secretion, biological actions and physiologic role. *Horm Res* 24:121–130, 1986
96. ROTWEIN P, POLLOCK KM, DIDIER DK, KRIVI GG: Organization and sequence of the human insulin-like growth factor I gene. *J Biol Chem* 261:4828–4832, 1986
97. BRISSENDEN JE, ULLRICH A, FRANCKE U: Human chromosomal mapping of genes for insulin-like growth factors I and II and epidermal growth factor. *Nature* 310:781–784, 1984
98. BORTZ JD, ROTWEIN P, DEVOL D, BECHTEL P, HANSEN VA, HAMMERMAN MR: Focal expression of insulin-like growth factor I in rat kidney collecting duct. *J Cell Biol* 107:811–819, 1988
99. ARON DC, HOUT WM, ABBOUD HE: Insulin-like growth factor I synthesis by human glomerular mesangial cells. (abstract) *Kidney Int* 35:308, 1989
100. HAMMERMAN MR: The growth hormone-insulin-like growth factor axis in kidney. *Am J Physiol* 257:F503–F514, 1989
101. HIRVONEN H, SANDBERG M, KALIMO H, HUKKANEN V, VUORIO E, SALMI TT, ALITALO K: The N-myc proto-oncogene and IGF-II growth factor mRNAs are expressed by distinct cells in human fetal kidney and brain. *J Cell Biol* 108:1093–1104, 1989
102. RECHLER MM, NISSLEY SP: The nature and regulation of the receptors for insulin-like growth factors. *Ann Rev Physiol* 47:425–442, 1985
103. HAMMERMAN MR, GAVIN JR: Binding of IGF I and IGF I-stimulated phosphorylation in canine renal basalateral membranes. *Am J Physiol* 251:E32–E41, 1986
104. CZECH MP: Signal transmission by the insulin-like growth factors. *Cell* 59:235–238, 1989
105. MACDONALD RG, PFEFFER SR, COUSSENS L, TEPPER MA, BROCKLEBANK CM, MOLE JE, ANDERSON JK, CHEN E, CZECH MP, ULLRICH A: A single receptor binds both insulin-like growth factor II and mannose-6-phosphate. *Science* 239:1134–1138, 1988
106. HAMMERMAN MR, ROGERS S: Distribution of IGF receptors in the plasma membrane of proximal tubular cells. *Am J Physiol* 253: F841–F847, 1987
107. HAMMERMAN MR, GAVIN JR: Binding of insulin-like growth factor II and multiplication-stimulating activity-stimulated phosphorylation in basolateral membranes from dog kidney. *J Biol Chem* 259:13511–13517, 1984
108. ANDERSSON G, JENNISCHE E: IGF-I immunoreactivity is expressed by regenerating renal tubular cells after ischaemic injury in rats. *Acta Physiol Scand* 132:453–457, 1988
109. STILES AD, SOSENKO IRS, D'ARCOLE AJ, SMITH BT: Relation of kidney tissue somatomedin-C/insulin-like growth factor I to postnephrectomy renal growth in the rat. *Endocrinology* 117:2397–2401, 1985
110. FLYVBJERG A, THORLACIUS-USSING O, NAERAA R, INGERSLEV J, ORSKOV H: Kidney tissue somatomedin C and initial growth in diabetic and uninephrectomized rats. *Diabetologia* 31:310–314, 1988
111. EL NAHAS AM, LE CARPENTIER JE, BASSETT AH, HILL DJ: Dietary protein and insulin-like growth factor-I content following unilateral nephrectomy. *Kidney Int* 36 (Suppl. 27):S15–S19, 1989
112. EL NAHAS AM, LE CARPENTIER JE, BASSETT AH: Compensatory renal growth: Role of growth hormone and insulin-like growth factor-I. *Nephrol Dial Trans* 5:123–129, 1990
113. FAGIN JA, MELMED S: Relative increase in insulin-like growth factor I messenger ribonucleic acid levels in compensatory renal hypertrophy. *Endocrinology* 120:718–724, 1987
114. LAJARA R, ROTWEIN P, BORTZ JD, HANSEN VA, SADOW JL, BETTS CR, ROGERS SA, HAMMERMAN MR: Dual regulation of insulin-like growth factor I expression during renal hypertrophy. *Am J Physiol* 257:F252–F261, 1989
115. CLEMMONS DR: Multiple hormones stimulate the production of somatomedin by cultured human fibroblast. *J Clin Endocr Metab* 58:850–856, 1984
116. CONOVER CA, DOLLAR LA, HINTZ RL, ROSENFELD RG: Insulin-like growth factor I/somatomedin-C (IGF-I/SM-C) and glucocorticoids synergistically regulate mitosis in competent human fibroblasts. *J Cell Physiol* 116:191–197, 1983
117. CONOVER CA, LIU F, POWELL D, ROSENFELD RG, HINTZ RL: Insulin-like growth factor binding proteins from cultured human fibroblast. *J Clin Invest* 83:852–859, 1989
118. DERYNCK R: Transforming growth factor alpha. *Cell* 54:593–595, 1988
119. LEE DC, ROSE TM, WEBB NR, TODARO GJ: Cloning and sequence analysis of a cDNA for rat transforming growth factor-α. *Nature* 313:489–191, 1985
120. DOWNWARD J, PARKER P, WATERFIELD MD: Autophosphorylation sites on the epidermal growth factor receptor. *Nature* 311: 483–485, 1984
121. BRACHMANN R, LINDQUIST PB, NAGASHIMA M, KOHR W, LIPARI T, NAPIER M, DERYNCK R: Transmembrane TGF-α precursors activate EGF/TGFα receptors. *Cell* 56:691–700, 1989
122. RAPPOLEE DA, MARK D, BANDA MJ, WERB Z: Wound macrophages express TGF-α and other growth factors in vivo: Analysis by mRNA phenotyping. *Science* 241:708–712, 1988
123. SALIDO EC, LAKSHMANAN J, BROWN P, ODELL W, BARAJAS L, FISHER D: Differential cellular localization of EGF and TGF-α in the rat kidney. (abstract) *Clin Res* 38:208A, 1990
124. REISS R, FUNKE AJ, CIESLINSKI DA, HUMES HD: Transforming growth factor alpha accelerates renal repair and recovery following ischemic injury to the kidney. (abstract) *Kidney Int* 37:492, 1990
125. SPORN MB, ROBERTS AB, WAKEFIELD LM, ASSOIAN RK: Transforming growth factor-β: Biological function and chemical structure. *Science* 233:532–534, 1986
126. MASSAGUE J, CHEIFETZ S, IGNOTZ RA, BOYD FT: Multiple type-b transforming growth factors and their receptors. *J Cell Physiol* (Suppl.)5:43–47, 1987
127. ROBERTS AB, FLANDERS KC, KONDAIAH P, THOMPSON NL, VAN OBBERGHEN-SCHILLING E, WAKEFIELD L, ROSSI P, DE CROMBRUGGHE B, HEINE U, SPORN MB: Transforming growth factor β: Biochemistry and roles in embryogenesis, tissue repair and remodeling, and carcinogenesis. *Rec Prog Horm Res* 44:157–197, 1988
128. ASSOIAN RK, KOMORIYA A, MEYERS CA, MILLER DM, SPORN MB: Transforming growth factor-beta in human platelets. Identification of a major storage site, purification and characterization. *J Biol Chem* 258:7155–7160, 1983
129. HOLLEY RW, BOEHLEN P, FAVA R, BALDWIN JH, KLEEMAN G, ARMOUR R: Purification of kidney epithelial cell growth inhibitors. *Proc Natl Acad Sci USA* 77:5989–5992, 1980
130. MASSAGUE J: The TGF-β family of growth and differentiation factors. *Cell* 49:437–438, 1987
131. MIYAZAKI K, HORIO T: Growth inhibitors: Molecular diversity and roles in cell proliferation. *In Vitro* 25:866–872, 1989
132. SPORN MB, ROBERTS AB, WAKEFIELD LM, DE CROMBRUGGHE B: Some recent advances in the chemistry and biology of transforming growth factor-beta. *J Cell Biol* 105:1039–1045, 1987
133. CHEIFETZ S, LIKE B, MASSAGUE J: Cellular distribution of type I and type II receptors for transforming growth factor-β. *J Biol Chem* 261:9972–9978, 1986
134. MOSES HL, COFFEY RJ, LEOF EB, LYONS RM, KESKI-OJA J: Transforming growth factor β regulation of cell proliferation. *J Cell Physiol* (Suppl.)5:1–7, 1987
135. MOSES HL, TUCKER RF, LEOF EB, COFFEY RJ, HALPER J, SHIPLEY GD: Type beta transforming growth factor is a growth stimulator and a growth inhibitor. *Cancer Cells* 3:65–71, 1985
136. CIESLINSKI DA, JOHNSON LB, BEALS T, HUMES HD: Cellular basis of transforming growth factor-beta antiproliferative effect on renal proximal tubule cells in primary culture. (abstract) *Kidney Int* 37:192, 1990
137. ROBERTS AB, SPORN MB, ASSOIAN RK, SMITH JM, ROCHE NS, WAKEFIELD LM, HEINE U, LIOTTA LA, FALANGA V, KEHRL JH, FAUCI AS: Transforming growth factor type β: Rapid induction in fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci USA* 83:4167–4171, 1986
138. PENTTINEN RP, KOBAYASHI S, BORNSTEIN P: Transforming growth factor β increases mRNA for matrix proteins both in the

presence and in the absence of changes in mRNA stability. *Proc Natl Acad Sci USA* 85:1105–1108, 1988
139. BORNSTEIN P, SAGE H: Regulation of collagen gene expression. *Prog Nucl Acid Res* 37:67–106, 1989
140. HEINO J, IGNOTZ RA, HEMLER ME, CROUSE C, MASSAGUE J: Regulation of cell adhesion receptors by transforming growth factor-β. *J Biol Chem* 264:380–388, 1989
141. BAIRD A, ESCH F, MORMEDE P, UENO N, LING N, BOEHLEN P, YING SY, WEHRENBERG WB, GUILLEMIN R: Molecular characterization of fibroblast growth factor; distribution and biological activities in various tissues. *Rec Progr Horm Res* 42:143–205, 1986
142. FLORKIEWICZ RZ, SOMMER A: Human basic fibroblast growth factor gene encodes four polypeptides: Three initiate translation from non-AUG codons. *Proc Natl Acad Sci USA* 86:3978–3981, 1989
143. LEE PL, JOHNSON DE, COUSENS LS, FRIED VA, WILLIAMS LT: Purification and complementary DNA cloning of a receptor for basic fibroblast growth factor. *Science* 245:57–60, 1989
144. MERGIA A, TISCHER E, GRAVES D, TUMOLO A, MILLER J, GOSPODAROWICZ D, ABRAHAM JA, SHIPLEY GD, FIDDES JC: Structural analysis of the gene for human acidic fibroblast growth factor. *Biochem Biophys Res Com* 164:1121–1129, 1989
145. RISAU W, EKBLOM P: Production of heparin-binding angiogenesis factor by the embryonic kidney. *J Cell Biol* 103:1101–1107, 1986
146. LOTSPEICH WD: Renal hypertrophy in metabolic acidosis and its relation to ammonia excretion. *Am J Physiol* 208:1135–1142, 1965
147. LOTSPEICH WD: Metabolic aspects of acid-base change. *Science* 155:1066–1075, 1967
148. GOLCHINI K, NORMAN J, BOHMAN R, KURTZ I: Induction of hypertrophy in cultured proximal tubule cells by extracellular NH_4Cl. *J Clin Invest* 84:1767–1779, 1989
149. ANITHAL HN, WALSH-REITZ MM, TOBACK FG: Appearance of a cytosolic protein that stimulates glyceraldehyde-3-phosphate dehydrogenase activity during initiation of renal epithelial cell growth. *Proc Natl Acad Sci USA* 80:2941–2945, 1983
150. MORDAN LJ, TOBACK FG: Growth of kidney epithelial cells in culture: Evidence for autocrine control. *Am J Physiol* 246:C351–C354, 1984
151. WALSH-REITZ MM, AITHAL HN, TOBACK FG: Na regulates growth in kidney epithelial cells induced by lowering extracellular K concentration. *Am J Physiol* 247:C321–C326, 1984
152. WALSH-REITZ MM, GLUCK SL, WAACK S, TOBACK FG: Lowering extracellular Na^+ concentration releases autocrine growth factors from renal epithelial cells. *Proc Natl Acad Sci USA* 83:4764–4768, 1986
153. KARTHA S, SUKHATME VP, TOBACK FG: ADP activates protooncogene expression in renal epithelial cells. *Am J Physiol* 252: F1175–F1179, 1987
154. HESSION C, DECKER JM, SHERBLOM AP, KUMAR S, YUE CC, MATTALIANO RJ, TIZARD R, KAWASHIMA E, SCHMEISSNER U, HELETKY S, CHOW EP, BURNE CA, SHAW A, MUCHMORE AV: Uromodulin (Tamm-Horsfall glycoprotein): A renal ligand for lymphokines. *Science* 237:1479–1484, 1987
155. ARMELIN HA, ARMELIN MCS: The interaction of peptide growth factors and oncogenes, in *Oncogenes, Genes and Growth Factors*, edited by GUROFF G, New York, John Wiley & Sons, 1987, pp. 331–373
156. MACARA IG: Oncogenes, ions, and phospholipids. *Am J Physiol* 248:C3–C11, 1985
157. SINKOVICS JG: Oncogenes and growth factors. *CRC Crit Rev Immunol* 8:217–298, 1988
158. SERUNIAN LA, CANTLEY LC: Growth factor and oncogene influences on cell growth regulation. *Ann NY Acad Sci* 551:309–319, 1988
159. MARCARA IG: Oncogenes and cellular signal transduction. *Physiol Rev* 69:797–820, 1989
160. FREISSMUTH M, CASEY PJ, GILMAN AG: G proteins control diverse pathways of transmembrane signaling. *FASEB J* 3:2125–2131, 1989
161. STRYER L, BOURNE HR: G Proteins: A family of signal transducers. *Ann Rev Cell Biol* 2:391–419, 1986
162. CASEY PJ, GILMAN AG: G protein involvement in receptor-effector coupling. *J Biol Chem* 263:2577–2580, 1988
163. BIRNBAUMER L, CODINA J, MATTERA R, YATANI A, SCHERER N, TORO MJ, BROWN AM: Signal transduction by G proteins. *Kidney Int* 32 (Suppl. 23):S14–S37, 1987
164. KOZASA T, ITOH H, TSUKAMOTO T, KAZIRO Y: Isolation and characterization of the human G_{sa} gene. *Proc Natl Acad Sci USA* 85:2081–2085, 1988
165. NEER EJ, CLAPHAM DE: Roles of G protein subunits in transmembrane signalling. *Nature* 333:129–134, 1988
166. GILMAN GA: G proteins: Transducers of receptor-generated signals. *Ann Rev Biochem* 56:615–649, 1987
167. GILMAN AG: G proteins and dual control of adenylate cyclase. *Cell* 36:577–579, 1984
168. RIBEIRO-NETO FAP, MATTERA R, HILDEBRANDT JD, CODINA J, FIELD JB, BIRNBAUMER L, SEKURA RD: ADP-ribosylation of membrane components by pertussis and cholera toxin. *Meth Enzymol* 109:566–572, 1985
169. ROZENGURT E: Early signals in the mitogenic response. *Science* 234:161–166, 1986
170. ROZENGURT E: Signal transduction pathways in mitogenesis. *Br Med Bull* 45:515–528, 1989
171. ROZENGURT E, STROOBNAT P, WATERFILED MD, DEUEL TF, KEEHAN M: Platelet-derived growth factor elicits cyclic AMP accumulation in Swiss 3T3 cells: Role of prostaglandin production. *Cell* 34:265–272, 1983
172. ESCRIBANO J, ROZENGURT E: Cyclic AMP increasing agents rapidly stimulate vimentin phosphorylation in quiescent cultures of Swiss 3T3 cells. *J Clin Invest* 137:223–234, 1988
173. MONTMINY MR, BILEZIKJIAN LM: Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. *Nature* 328:175–178, 1987
174. SASSONE-CORSI P: Cyclic AMP induction of early adenovirus promotors involves sequences required for E1A trans-activation. *Proc Natl Acad Sci USA* 85:7192–7196, 1988
175. SWINNEN JV, JOSEPH DR, CONTI M: The mRNA encoding a high-affinity cAMP phosphodiesterase is regulated by hormones and cAMP. *Proc Natl Acad Sci USA* 86:8197–8201, 1989
176. MILANES CL, PERNALETE N, STAROSTA R, PEREZ-GONZALEZ M, PAZ-MARTINEZ V, BELLORIN-FONT E: Altered response of adenylate cyclase to parathyroid hormone during compensatory renal growth. *Kidney Int* 26:802–809, 1989
177. BLACKSHEAR PJ: Converging and diverging pathways in polypeptide hormone action. *Clin Res* 37:554–563, 1989
178. ROGERS SA, HAMMERMAN MR: Growth hormone activates phospholipase C in proximal tubular basolateral membranes from canine kidney. *Proc Natl Acad Sci USA* 86:6363–6366, 1989
179. COCKCROFT S, STUTCHFIELD J: G-protein, the inositol lipid signalling pathway, and secretion. *Phil Trans R Soc Lond* B320:411–416, 1988
180. ZACHARY I, MILLAR J, NANBERG E, HIGGINS T, ROZENGURT E: Inhibition of bombesin-induced mitogenesis by pertussis toxin: Dissociation from phospholipase C pathway. *Biochem Biophys Res Com* 146:456–463, 1987
181. PFEILSCHIFTER J, BAUER C: Pertussis toxin abolishes angiotensin II-induced phosphoinositide hydrolysis and prostaglandin synthesis in rat renal mesangial cells. *Biochem J* 236:289–294, 1986
182. PFEILSCHIFTER JM: Cellular signalling in the kidney: The role of inositol lipids. *Renal Physiol Biochem* 12:1–31, 1989
183. SANTOS E, NEBREDA AR: Structural and functional properties of ras proteins. *FASEB J* 3:2151–2163, 1989
184. MCCORMICK F: Ras GTPase activating protein: Signal transmitter and signal terminator. *Cell* 56:5–8, 1989
185. BARBACID M: Ras genes. *Ann Rev Biochem* 56:779–827, 1987
186. YO CL, TSAI MH, STACEY DW: Cellular ras activity and phospholipid metabolism. *Cell* 52:63–71, 1988
187. RHEE SG, SUH PG, RYU SH, LEE SY: Studies of inositol phospholipid-specific phospholipase C. *Science* 244:546–550, 1989
188. BOYER JL, HEPLER JR, HARDEN TK: Hormone and growth factor receptor-mediated regulation of phospholipase C activity. *TIPS* 10:360–364, 1989
189. KUMJIAN DA, WAHL MI, RHEE SG, DANIEL TO: Platelet-derived growth factor (PDGF) binding promotes physical association of PDGF receptor with phospholipase C. *Proc Natl Acad Sci USA* 86:8232–8236, 1989

190. BERRIDGE MJ, IRVINE RF: Inositol triphosphate, a novel second messenger in cellular signal transduction. *Nature* 312:315–321, 1984
191. EXTON JH: Calcium signalling in cells—molecular mechanisms. *Kidney Int* 32 (Suppl. 23):S68–S76, 1987
192. WANG JH, PALLEN CJ, SHARMA RK, ADACHI AM, ADACHI K: The calmodulin regulatory system. *Curr Top Cell Reg* 27:419–436, 1985
193. BERRIDGE MJ: Inositol lipids and cell proliferation, in *Oncogenes and Growth Control*, edited by KAHN P, GRAF T, Berlin, Springer-Verlag, 1986, pp. 147–153
194. NISHIZUKA Y: The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* 308:693–698, 1984
195. NISHIZUKA Y: Studies and perspectives of protein kinase C. *Science* 233:305–312, 1986
196. NEIDEL JE, KUHN LJ, VANDENBARK GR: Phorbol diester receptor copurifies with protein kinase C. *Proc Natl Acad Sci USA* 80:36–40, 1983
197. NISHIZUKA Y: The molecular heterogeinity of protein kinase C and its implications for cellular regulation. *Nature* 334:661–665, 1988
198. COUSSENS L, PARKER PJ, RHEE L, YANG-FENG TL, CHEN E, WATERFIELD MD, ULLRICH A: Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science* 233:859–866, 1986
199. ONO Y, FUJII T, IGARASHI K, KUNO T, TANAKA C, KIKKAWA U, NISHIZUKA Y: Phorbol ester binding to protein kinase C requires cysteine-zinc finger-like sequence. *Proc Natl Acad Sci USA* 86:4868–4871, 1989
200. STRUHL K: Helix-turn-helix, zinc-finger, and leucine-zipper motifs for eukaryotic transcriptional regulatory proteins. *TIBS* 14: 137–140, 1989
201. BARRETT PQ, ZAWALICH K, RASMUSSEN H: Protein kinase C activity in renal microvillus membranes. *Biochem Biophys Res Com* 128:494–505, 1985
202. BONI LT, RANDO RR: The nature of protein kinase C activation by physically defined phospholipid vesicles and diacylglcerols. *J Biol Chem* 260:10819–10825, 1985
203. BERRIDGE MJ, HESLOP JP, IRVINE RF, BROWN KD: Inositol triphosphate formation and calcium mobilization in Swiss 3T3 cells in response to platelet-derived growth factor. *Biochem J* 222:195–201, 1984
204. ERUSALIMSKY JG, ROZENGURT E: Vasopressin rapidly stimulates protein kinase C in digitonin-permeabilized Swiss 3T3 cells: Involvement of a pertussis toxin-insensitive guanine nucleotide binding protein. *J Cell Physiol* 141:253–261, 1989
205. KAPLAN DR, WHITMAN M, SCHAFFHAUSEN B, PALLAS DC, WHITE M, CANTLEY L, ROBERTS TM: Common elements in growth factor stimulation and oncogenic transformation: 85 Kd phosphoprotein and phosphatidylinositol kinase activity. *Cell* 50: 1021–1029, 1987
206. HISE MK, MEHTA PS: Activity of calcium-sensitive phospholipid-dependent protein kinase C followin nephron loss. *Kidney Int* 36:216–221, 1989
207. SALIHAGIC A, MACKOVIC M, BANFIC H, SABOLIC I: Short-term and long-term stimulation of Na^+-H^+ exchange in cortical brush-border membranes during compensatory growth of the rat kidney. *Eur J Physiol* 413:190–196, 1988
208. WEDEGAERTNER PB, GILL GN: Activation of the purified protein tyrosine kinase domain of the epidermal growth factor receptor. *J Biol Chem* 264:11346–11353, 1989
209. CHEN WS, LAZAR CS, LUND KA, WELSH JB, CHANG CP, WALTON GM, DER CJ, WILEY HS, GILL GN, ROSENFELD MG: Functional independence of the epidermal growth factor receptor from a domain required for ligand-induced internalization and calcium regulation. *Cell* 59:33–43, 1989
210. SIBLEY DR, BENOVIC JL, CARON MG, LEFKOWITZ RJ: Regulation of transmembrane signalling by receptor phsophorylation. *Cell* 48:913–922, 1987
211. SEFTON BM, HUNTER T: Tyrosine protein kinases. *Adv Cyclic Nucl Protein Phosph Res* 18:195–226, 1984
212. BEN-NERIAH Y, BERNARDS A, PASKIND M, DALEY GO, BALTIMORE D: Alternative 5'exons in c-abl mRNA. *Cell* 44:577–587, 1986
213. HUNTER T: A thousand and one kinases. *Cell* 50:823–829, 1987
214. NOVAK-HOFER I, THOMAS G: Epidermal growth factor-mediated activation of an S 6 kinase in Swiss mouse 3T3 cells. *J Biol Chem* 260:10314–10319, 1985
215. JENOE P, JAEGGI N, LUTHER H, SIEGMANN M, THOMAS G: Purification and characterization of a 40S ribosomal protein S6 kinase from vanadate-stimulated Swiss 3T3 cells. *J Biol Chem* 264:1293–1297, 1989
216. SUSA M, OLIVIER AR, FABBRO D, THOMAS G: EGF induces biphasic S6 kinase activation: Late phase is protein kinase C-dependent and contributes to mitogeneicity. *Cell* 57:817–824, 1989
217. MCKANNA JA, CHUNCHARUNEE A, BREYER J, HARRIS RC: Localization of the calcium-dependent 35 kDa substrate for epidermal growth factor receptor kinase (p35) in rat kidney. (abstract) *Kidney Int* 37:374, 1990
218. HARRIS RC: S6 kinase activity increases in cultured proximal tubule cells with epidermal growth factor and insulin and in renal cortex after uninephrectomy. (abstract) *Kidney Int* 37:195, 1990
219. KAHN CR, WHITE MF: The insulin receptor and the molecular mechanim of insulin action. *J Clin Invest* 82:1151–1156, 1988
220. CYERT MS, THORNER J: Putting it on and taking it off: Phosphoprotein phosphatase involvement in cell cycle regulation. *Cell* 57:891–893, 1989
221. PFEILSCHIFTER J: Cross-talk between transmembrane signalling systems: A prerequisite for the delicate regulation of glomerular haemodynamics by mesangial cells. *Eur J Clin Invest* 19:347–361, 1989
222. ROZENGURT E, MURRAY M, ZACHARY I, COLLINS M: Protein kinase C activation enhances cAMP accumulation in Swiss 3T3 cells: Inhibition by pertussis toxin. *Proc Natl Acad Sci USA* 84:2282–2286, 1987
223. KATO H, ISHITOYA J, TAKENAWA T: Inhibition of inositol phospholipids metabolism and calcium mobilization by cyclic AMP-increasing agents and phorbol ester in neutrophils. *Biochem Biophys Res Com* 139:1272–1278, 1986
224. GRAFF JM, STUMPO DJ, BLACKSHEAR PJ: Characterization of the phsphorylation sites in the chicken and bovine myristoylated alanine-rich C kinase substrate (MARCKS) protein, a prominent cellular substrate for protein kinase C. *J Biol Chem* 264:11912–11919, 1989
225. MOOLENAAR WH: Effects of growth factors on intracellular pH regulation. *Ann Rev Physiol* 48:363–376, 1986
226. CASSEL D, ROTHENBERG P, WHITELEY B, MANCUSO D, SCHLESSINGER P, REUSS L, CRAGOE EJ, GLASER L: Control of mitogenic activation of Na^+-H^+ exchange. *Curr Topics Membr Trans* 26: 157–173, 1986
227. GRINSTEIN S, ROTIN D, MASON MJ: Na^+/H^+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. *Biochem Biophys Acta* 988:73–97, 1989
228. GRINSTEIN S, COHEN S, GOETZ JD, ROTHSTEIN A, GELFAND EW: Characterization of Na^+/H^+ exchange in lymphocytes by phorbol ester. Change in the cytoplasmic pH dependance of the antiport. *Proc Natl Acad Sci USA* 82:1429–1433, 1985
229. WEIMAN EJ, SHENOLIKAR S, KAHN AM: cAMP-associated inhibition of Na^+/H^+ exchanger in rabbit kidney brush border membranes. *Am J Physiol* 252:F19–F25, 1987
230. WEINMAN EJ, DUBINSKY W, SHENOLIKAR S: Regulation of the renal Na^+-H^+ exchanger by protein phosphorylation. *Kidney Int* 36:519–525, 1989
231. KINSELLA J, SACKTOR B: Thyroid hormones increases Na^+-H^+ exchange activity in renal brush border membranes. *Proc Natl Acad Sci USA* 82:3606–3610, 1985
232. MELLAS J, GAVIN JR, HAMMERMAN MR: Multiplication stimulating activity-induced alkalinization of canine renal proximal tubular cells. *J Biol Chem* 261:14437–14442, 1986
233. MACKOVIC-BASIC M, FAN R, KURTZ I: Renal nerves mediate the early increase in Na^+/H^+ antiport activity following uninephrectomy. (abstract) *Kidney Int* 37:200, 1990
234. SEIFTER JL, ARONSON PS: Properties and physiologic roles of the plasma membrane sodium-hydrogen exchanger. *J Clin Invest* 78:859–864, 1986

235. BESTERMAN JM, TYREY SJ, CRAGOE EJ, CUATRECASA P: Inhibition of epidermal growth factor-induced mitogenesis by amiloride and an analog: Evidence against a requirement for Na^+/H^+ exchange. *Proc Natl Acad Sci USA* 81:6762–6766, 1984
236. LUBIN M, CAHN F, COUNTERMARSH BA: Amiloride, protein synthesis, and activation of quiescent cells. *J Cell Physiol* 113: 247–251, 1982
237. GRANTHAM JJ, GRANTHAM JA, DONOSO VS, CRAGOE EJ: Effect of amiloride on the compensatory renal growth that follows uninephrectomy in mice. *J Lab Clin Med* 113:129–134, 1989
238. MOHRMANN M, CANTIELLO HF, AUSIELLO DA: Renal epithelial cell growth can occur in absence of Na^+-H^+ exchanger activity. *Am J Physiol* 253:C633–C638, 1987
239. FAN R, MACKOVIC-BASIC M, KURTZ I, FINE LG: Unravelling cause and effect in renal hypertrophy: Absence of an essential initiating role for increased Na^+/H^+ exchange activity. (abstract) *Kidney Int* 37:193, 1990
240. KRUIJER W, COOPER JA, HUNTER T, VERMA IM: Platelet-drived growth factors induces rapid but transient expression of the c-fos gene and protein. *Nature* 312:711–716, 1984
241. MUELLER R, BRAVO R, BURCKHARDT J, CURRAN T: Induction of c-fos gene and protein by growth factors precedes activation of c-myc. *Nature* 312:716–720, 1984
242. QUANTIN B, BREATHNACH R: Epidermal growth factor stimulates transcription of the c-jun proto-oncogene in rat fibroblasts. *Nature* 334:538–539, 1988
243. SUKHATME VP, KARTHA S, TOBACK FG, TAUB R, HOOVER RG, TSAI-MORRIS CH: A novel early growth response gene rapidly induced by fibroblast, epithelial cell and lymphocyte mitogens. *Oncogene Res* 1:343–355, 1987
244. RYSECK RP, MACDONALD-BRAVO H, MATTEI MG, BRAVO R: Cloning and sequence of a secretory protein induced by growth factors in mouse fibroblasts. *Exp Cell Res* 180:266–275, 1989
245. ALMENDRAL JM, SOMMER D, MACDONALD-BRAVO H, BURCKHARDT J, PERERA J, BRAVO R: Complexity of the early genetic response to growth factors in mouse fibroblasts. *Mol Cell Biol* 8:2140–2148, 1988
246. RITTLING SR, GIBSON CW, FERRARI S, BASERGA R: The effect of cycloheximide on the expression of cell cycle dependent genes. *Biochem Biophys Res Com* 132:327–335, 1985
247. MILLER AD, CURRAN T, VERMA IM: C-fos protein can induce cellular transformation: A novel mechanism of activation of a cellular oncogene. *Cell* 36:51–60, 1984
248. GILMAN MZ, WILSON RN, WEINBERG RA: Multiple protein-binding sites in the 5′-flanking region regulate c-fos expression. *Mol Cell Biol* 6:4305–4316, 1986
249. FORT P, RECH J, VIE A, PIECHACZYK M, BONNIEU A, JEANTEUR P, BLANCHARD JM: Regulation of c-fos gene expression in hamster fibroblast: Initiation and elongation of transcription and mrNA dgradation. *Nucl Acid Res* 15:5657–5667, 1987
250. SASSONE-CORSI P, SISSON JC, VERMA IM: Transcriptional autoregulation of the proto-oncogene fos. *Nature* 334:314–319, 1988
251. SASSONE-CORSI P, VERMA IM: Modulation of c-fos gene transcription by negative and positive cellular factors. *Nature* 326:507–510, 1987
252. CHIU R, BOYLE WJ, MEEK J, SMEAL T, HUNTER T, KARIN M: The c-fos protein interacts with c-jun/AP-1 to stimulate transcription of AP-1 responsive genes. *Cell* 54:541–552, 1988
253. TURNER R, TJIAN R: Leucine repeats and an adjacent DNA binding domain mediate the formation of functional cfos-cjun heterodimers. *Science* 243:1689–1694, 1989
254. FRANZA BR, RAUSCHER FJ, JOSEPHS SF, CURRAN T: The fos complex and fos-related antigens recognize sequence elements that contain AP-1 binding sites. *Science* 239:1150–1153, 1988
255. GENTZ R, RAUSCHER FJ, ABATE C, CURRAN T: Parallel association of fos and jun leucine zippers juxtaposes DNA binding domains. *Science* 243:1695–1699, 1989
256. NAKABEPPU Y, NATHANS D: The basic region of fos mediates specific DNA binding. *EMBO J* 8:3833–3841, 1989
257. RISSE G, JOOSS K, NEUBERG M, BRUELLER HJ, MUELLER R: Asymetrical recognition of the palindromic AP 1 binding site (TRE) by fos protein complexes. *EMBO J* 8:3825–3832, 1989
258. VOGT PK, TJIAN R: Jun: A transcriptional regulator turned oncogenic. *Oncogene* 3:3–7, 1988
259. QUANTIN B, BREATHNACH R: Epidermal growth factor stimulates transcription of the c-jun proto-oncogene in rat fibroblast. *Nature* 334:538–539, 1988
260. ANGEL P, HATTORI K, SMEAL T, KARIN M: The jun proto-oncogene is positively autoregulated by its product, Jun/AP-1. *Cell* 55:875–885, 1988
261. RYSECK RP, HIRAJ SI, YANIV M, BRAVO R: Transcriptional activation of c-jun during the G_0-G_1 transition in mouse fibroblasts. *Nature* 334:535–537, 1988
262. ZERIAL M, TOSCHI L, RYSECK RP, SCHUERMANN M, MUELLER R, BRAVO R: The product of a novel growth factor activated gene, fos B, interacts with jun proteins enhancing their DNA binding activitz. *EMBO J* 8:805–813, 1989
263. GAZIN C, DUPONT DE DINECHIN S, HAMPE A, MASSON JM, MARTIN P, STEHELIN D, GALIBERT F: Nucleotide sequence of the human c-myc locus: Provocative open reading frame within the first exon. *EMBO J* 3:383–387, 1984
264. LIPP M, SCHILLING R, WIEST S, LAUX G, BORNKAMM GW: Target sequences for cis-acting regulation within the dual promotor of the human c-myc gene. *Mol Cell Biol* 7:1393–1400, 1987
265. ASSELIN C, NEPVEU A, MARCU KB: Molecular requirements for transcriptional initiation of the murine c-myc gene. *Oncogene* 4:549–558, 1989
266. NEPVEU A, LEVINE RA, CAMPISI J, GREENBERG ME, ZIFF EB, MARCU KB: Alternative modes of c-myc regulation in growth factor-stimulated and differentiating cells. *Oncogene* 1:243–250, 1987
267. LINSTEN T, JUNE CH, THOMPSON CB: Multiple mechanism regulate c-myc expression during normal T cell activation. *EMBO J* 7:2787–2794, 1988
268. COWLEY BD, SMARDO FL, GRANTHAM JJ, CALVET JP: Elevated c-myc proto-oncogene expression in autosomal recessive polycystic kidney disease. *Proc Natl Acad Sci USA* 84:8394–8398, 1987
269. TRUDEL M, D'AGATI V, CONSTANTINI F: The c-myc oncogene induces kidney cysts in transgenic mice. (abstract) *Kidney Int* 35:364, 1989
270. ASSELIN C, MARCU KB: Mode of c-myc gene regulation in folic acid-induced kidney regeneration. *Oncogene Res* 5:67–72, 1989
271. COWLEY BD, CHADWICK JJ, GRANTHAM JJ, CALVET JP: Sequential proto-oncogene expression in regenerating kidney following acute renal injury. *J Biol Chem* 264:8389–8393, 1989
272. HUMES HD, DANIEL TO: Proto-oncogene and growth factor gene expression during the repair phase of ischemic acute renal failure. (abstract) *Kidney Int* 37:484, 1990
273. BEER DG, ZWEIFEL KA, SIMPSON DP, PITOT HC: Specific gene expression during compensatory renal hypertrophy in the rat. *J Cell Physiol* 131:29–35, 1987
274. NORMAN JT, BOHMAN RE, FISCHMANN G, BOWEN JW, MCDONOUGH A, SLAMON D, FINE LG: Patterns of mRNA expression during early cell growth differ in kidney epithelial cell destined to undergo compensatory hypertrophy versus regenerative hyperplasia. *Proc Natl Acad Sci USA* 85:6768–6772, 1988
275. SAWCZUK IS, OLSSON CA, BUTTYAN R, NGUYEN-HUU MC, ZIMMERMAN KA, ALT FW, WOLGEMUTH D, REITELMAN C: Gene expression in renal growth and regrowth. *J Urol* 140:1145–1148, 1988
276. SAWCZUK IS, HOKE G, OLSSON CA, CONNOR J, BUTTYAN R: Gene expression in response to acute unilateral ureteral obstruction. *Kidney Int* 35:1315–1319, 1989
277. ZELMAN SJ, ZENSER TV, DAVIS BB: Renal growth in response to unilateral obstruction. *Kidney Int* 23:594–598, 1983
278. OUELLETTE AJ, MALT RA, SUKHATME VP, BONVENTRE JV: Expression of two "immediate early" genes, Egr-1 and c-fos, in response to renal ischemia and during compensatory renal hypertrophy in mice. *J Clin Invest* 85:766–771, 1990
279. SCHLONDORFF D, TRIZNA W, DE ROSIS E, KORTH-SCHULZ S: Effect of testosterone on compensatory renal hypertrophy in the rat. *Endocrinology* 101:1670–1675, 1977
280. BERGER FG, WATSON G: Androgen-regulated gene expression. *Ann Rev Physiol* 51:51–65, 1989
281. STEPHAN F, REVILLE P, DE LAHARPE F, KOELL-BACK MH:

Impairment of renal compensatory hypertrophy by hypothyroidism. *Life Sci* 30:623–631, 1982
282. Samuels HH, Forman BM, Horowitz ZD, Ye ZS: Regulation of gene expression by thyroid hormone. *J Clin Invest* 81:957–967, 1988
283. Evans EM: The steroid and thyroid hormone receptor superfamily. *Science* 240:889–895, 1988
284. Beato M: Gene regulation by steroid hormones. *Cell* 56:335–344, 1989
285. Chang C, Kokontis J, Liao S: Molecular cloning of the human and rat cDNA encoding androgen receptors. *Science* 240:324–326, 1988
286. Yamamoto KR: Steroid receptor regulated transcription of specific genes and gene network. *Ann Rev Genet* 19:209–215, 1985
287. Kumar V, Green S, Stack G, Berry M, Jin JR, Chambon P: Functional domains of the human estrogen receptor. *Cell* 51:941–951, 1987
288. Green S, Kumar V, Theulaz I, Wahli W, Chambon P: The N-terminal DNA-binding "zinc finger" of the oestrogen and glucocortioid receptor determines target gene specificity. *EMBO J* 7:3037–3044, 1988
289. Schleif R: DNA binding by proteins. *Science* 241:1182–1187, 1988
290. Ham J, Thomson A, Needham M, Webb P, Parker M: Characterization of response elements for androgens, glucocorticoids in mouse mammary tumour virus. *Nucl Acid Res* 16:5263–5276, 1988
291. Kandala JC, Kistler MK, Kistler WS: Androgen regulated genes from prostate and seminal vesicle share upstream sequence homologies. *Biochem Biophys Res Com* 126:948–952, 1985
292. Rheaume C, Barbour KW, Tseng-Crank J, Berger FG: Molecular genetics of androgen-inducible RP2 gene transcription in the mouse kidney. *Mol Cell Biol* 9:477–483, 1989
293. Berger FG, Watson G: Androgen induction of messenger RNA concentrations in mouse kidney is posttranscriptional. *Biochem* 25:1170–1175, 1986
294. Jetten MA: Retinoids specifically enhance the number of epidermal growth factor receptors. *Nature* 284:626–629, 1980
295. Jetten AM, Goldfarb RH: Action of epidermal growth factor and retinoids on anchorage-dependent and -independent growth of nontransformed ratd kidney cells. *Cancer Res* 43:2094–2099, 1983
296. Argile A, Kraft NE, Hutchinson P, Senes-Ferrari S, Atkins RC: Retinoic acid affects the cell cycle and increases total protein content in epithelial cells. *Kidney Int* 36:954–959, 1989
297. Taub M: Retinoic acid modulates dome formation by MDCK cells in defined medium. *J Cell Biol* 141:24–32, 1989
298. Argiles A, Mourad G, Basset N, Axelrud-Cavadore C, Haiech J, Mion C, Cavadore JC, Demaille JG: Acute adaptive changes to unilateral nephrectomy in humans. *Kidney Int* 32:714–720, 1987
299. Bedo G, Santisteban P, Aranda A: Retinoic acid regulates growth hormone gene expression. *Nature* 339:231–234, 1989
300. Umesono K, Giguere V, Glass CK, Rosenfeld MG, Evans RM: Retinoic acid and thyroid hormone induce gene expression through a common responsive element. *Nature* 336:262–265, 1988
301. Ouellette AJ: Messenger mRNA regulation during compensatory renal growth. *Kidney Int* 23:575–580, 1983
302. Ouellette AJ, Malt RA: Noncoordinate regulation of cytoplasmic RNA in compensatory renal hypertrophy. *Am J Physiol* 237:R360–R365, 1979
303. Ouellette AJ, Ordahl CP: Extensive homology between poly(A)-containing mRNA and purified nominal poly(A)-lacking mRNA in mouse kidney. *J Biol Chem* 256:5104–5108, 1981
304. Miller WL, McCarthy BJ: Gene expression in normal and regenerating mouse kidney. *J Biol Chem* 254:742–748, 1979
305. Ouellette AJ, Moonka R, Zelenetz AD, Malt RA: Regulation of ribosome synthesis during compensatory renal hypertrophy in mice. *Am J Physiol* 253:C506–C513, 1987
306. Hargrove JL, Schmidt FH: The role of mRNA and protein stability in gene expression. *FASEB J* 3:2360–2370, 1989
307. Schneider C, King RM, Philipson L: Genes specifically expressed at growth arrest of mammalian cells. *Cell* 54:787–793, 1988
308. McClung JK, Danner DB, Stewart DA, Smith JR, Schneider EL, Lumpkin CK, Dell'Orco RT, Nuell MJ: Isolation of a cDNA that hybrid selects antiproliferative mRNA from rat liver. *Biochem Biophys Res Com* 164:1316–1322, 1989
309. Toback GF, Smith PD, Lowenstein LM: Phospholipid metabolism in the initiation of renal compensatory growth after acute reduction of renal mass. *J Clin Invest* 54:91–97, 1974
310. Hise MK, Harris RH, Mansbach CM: Regulation of de novo phosphatidylcholine biosynthesis during renal growth. *Am J Physiol* 247:F260–F266, 1984
311. Toback FG, Havener LJ, Dodd RC, Spargo BH: Phospholipid metabolism during renal regeneration after acute tubular necrosis. *Am J Physiol* 232:E216–E222, 1977
312. Humphreys MH, Etheredge SB, Lin SY, Ribstein J, Marton LJ: Renal ornithine decarboxylase activity, polyamines, and compensatory renal hypertrophy in the rat. *Am J Physiol* 255:F270–F277, 1988
313. Salehmoghaddam S, Bradley T, Mikhail N, Badie-Dezfooly B, Nord EP, Trizna W, Kheyfets R, Fine LG: Hypertrophy of basolateral Na-K pump activity in the proximal tubule of the remnant kidney. *Lab Invest* 53:443–452, 1985
314. Jacobson MP, Rodriguez HJ, Hogan WC, Klahr S: Mechanism of activation of renal Na^+-K^+-ATPase in the rat: Effects of reduction of renal mass. *Am J Physiol* 239:F281–F288, 1980
315. Wolf G, Thaiss F, Scherberich JE, Schoeppe W, Stahl RAK: Glomerular angiotensinase A in the rat: Increase of enzyme activity following renal ablation. *Kidney Int* 38:862–868, 1990
316. Hoang T, Bergeron M: Segmental heterogeneity of enzymatic response during compensatory renal growth. *Growth* 49:439–449, 1985
317. Harris DCH, Chan L, Schrier RW: Remnant kidney hypermetabolism and progression of chronic renal failure. *Am J Physiol* 254:F267–F276, 1988
318. Stahl RAK, Kudelka S, Paravicini M, Schollmeyer P: Prostaglandin and thromboxane formation in glomeruli from rats with reduced renal mass. *Nephron* 42:252–257, 1986
319. Stahl RAK, Kudelka S, Helmchen U: High protein intake stimulates glomerular prostaglandin formation in remnant kidneys. *Am J Physiol* 252:F1093–F1094, 1987
320. Logan JL, Lee SM, Benson B, Michael UF: Inhibition of compensatory renal growth by indomethacin. *Prostaglandins* 31: 253–261, 1986
321. Purkerson ML, Joist JH, Yates J, Valdes A, Morrison A, Klahr S: Inhibition of thromboxane synthesis ameliorates the progressive kidney disease of rats with subtotal renal ablation. *Proc Natl Acad Sci USA* 82:193–197, 1985